STIC Search Report
EIC 3700

STIC Database Tracking Number: 124402

TO: Samuel Gilbert **Location: cp2 4 d25** **Art Unit: 3736** **Tuesday, June 15, 2004** **Case Serial Number: 10/760646**	**From: Emory Damron** **Location: EIC 3700** **CP2-2C08** **Phone: 305-8587** **Emory.Damron@uspto.gov**

Search Notes

Dear Samuel,

Please find below an inventor search in the bibliographic and full-text foreign patent files, as well as keyword searches in the patent and non-patent literature files, both bibliographic and full text.
References of potential pertinence have been tagged, but please review all the packets in case you like something I didn't.
In addition to searching on Dialog, I also searched Google.com, EPO/JPO/Derwent, ScienceDirect and Scirus.com.
The very closest reference I found was to the applicant, US 6679827; claim 1 in that patent and claim 1 in this case, as you are probably aware, are very similar.
My next favorite reference is WO 9966987, to Tofani. I also included quite a bit of nonpatent literature, all published before the priority filing date of this case.
Please contact me if I can refocus or expand any aspect of this case, and please take a moment to provide any feedback (on the form provided) so EIC 3700 may better serve your needs.
Sincerely,

Emory Damron
Technical Information Specialist
EIC 3700, US Patent & Trademark Office
Phone: (703) 305-8587/ Fax: (703) 306-5915
Emory.damron@uspto.gov

Access DB# 124402

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Samuel Gilbert Examiner #: 70632 Date: 6/10/04
Art Unit: 3736 Phone Number 308-3553 Serial Number: 10/760,646
Mail Box and Bldg/Room Location: CP2 4B25 Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Magnetic Field Enhancement of Tumor Treatment

Inventors (please provide full names): Robert Sandstrom

Earliest Priority Filing Date: 10/11/2001

**For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

See attached claims.

method of treating a tumor as set forth in claim 1.

****

STAFF USE ONLY

Searcher: Dawson, Emory
Searcher Phone #: 305-8587
Searcher Location: CP2, 7C8
Date Searcher Picked Up: 6/15/04 9AM
Date Completed: 6/15/04 2:15P
Searcher Prep & Review Time: 185m
Clerical Prep Time: 0
Online Time: 185m

Type of Search

NA Sequence (#)
AA Sequence (#)
Structure (#)
Bibliographic X
Litigation
Fulltext X
Patent Family
Other

Vendors and cost where applicable

STN
Dialog X 1002.79
Questel/Orbit
Dr.Link
Lexis/Nexis
Sequence Systems
WWW/Internet X Scirus/ScienceDirect
Other (specify)

PTO-1590 (8-01)

STIC Search Results Feedback Form

EIC 3700

Questions about the scope or the results of the search? Contact ***the EIC searcher or contact:***

John Sims, EIC 3700 Team Leader
308-4836, CP2-2C08

Voluntary Results Feedback Form

- *I am an examiner in Workgroup:* 3736 *Example: 3730*
- *Relevant prior art **found**, search results used as follows:*
  - ☐ 102 rejection
  - ☐ 103 rejection
  - ☐ Cited as being of interest.
  - ☐ Helped examiner better understand the invention.
  - ☐ Helped examiner better understand the state of the art in their technology.

  Types of relevant prior art found:
  - ☐ Foreign Patent(s)
  - ☐ Non-Patent Literature
    (journal articles, conference proceedings, new product announcements etc.)
- *Relevant prior art **not found:***
  - ☐ Results verified the lack of relevant prior art (helped determine patentability).
  - ☐ Results were not useful in determining patentability or understanding the invention.

Comments:

Drop off or send completed forms to STIC/EIC3700 CP2 2C08

```
Set      Items   Description
S1      180822   (MAGNET? OR ELECTROMAGNET? OR PARAMAGNET? OR FERROMAGNET?)-
              (3N)(FIELD? OR SPHERE? OR AREA? OR REGION? OR ZONE? OR PENUMB-
              R?)
S2       19733   MRI OR MAGNET?()RESONAN?
S3      110655   CANCER? OR TUMOR? OR TUMOUR? OR MALIGNAN? OR NEOPLAS? OR N-
              EO()PLAS? OR CARCINO?
S4       18223   FREE()RADICAL? OR OXYGEN()RADICAL?
S5          61   ATOM?(5N)(UNPAIR? OR UNMATCH? OR LOST)(2N)ELECTRON? ?
S6       13581   APOPTO? OR NECROSIS? OR NECROTI? OR NECROBIO? OR TUMORCID?
              OR TUMOURCID?
S7        6620   (CELL? ? OR CELLULAR?)(2N)(DEATH? OR DIE OR DIES OR DIED OR
               DYING OR KILL? OR SUICID?)
S8       14986   (THERAPY? OR THERAPEUT? OR THERAPIE? OR TREATMENT?)(3N)(RA-
              DIAT? OR GAMMA? OR GAMMARAY? OR SOUND? OR ACOUSTIC? OR ULTRAS-
              OUND? OR ULTRASON? OR ULTRA()(SONIC? OR SOUND?) OR PHOTO? OR -
              XRAY? OR X()(RAY OR RAYS OR RAYING OR RAYED))
S9     4129401   METHOD? ?
S10    3008436   SYSTEM? ?
S11    2434808   PROCESS??
S12     199639   PROCEDURE? ?
S13     223146   TECHNIQUE? ?
S14     358299   IC=(A61B? OR A61N? OR A61M?)
S15          6   S1 AND S3 AND S4:S5
S16          1   S15 AND S6:S7
S17          6   S15 AND S8:S14
S18          6   S15:S17
S19          6   IDPAT (sorted in duplicate/non-duplicate order)
? show files
File 347:JAPIO Nov 1976-2004/Feb(Updated 040607)
         (c) 2004 JPO & JAPIO
File 350:Derwent WPIX 1963-2004/UD,UM &UP=200437
         (c) 2004  Thomson Derwent
?
```

APPLICANT

19/3,K/1 (Item 1 from file: 350)
DIALOG(R)File 350:Derwent WPIX

015320734 **Image available**
WPI Acc No: 2003-381669/200336
XRAM Acc No: C03-101420
XRPX Acc No: N03-304865

Treatment of tumor **involves creating elevated concentration of** free radicals **in** tumor , **and creating** magnetic field **that traverses** tumor **and inhibits recombination of** free radicals **in** tumor

Patent Assignee: SANDSTROM R E (SAND-I)
Inventor: SANDSTROM R E
Number of Countries: 097 Number of Patents: 003
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
WO 200330722	A2	20030417	WO 2002US32444	A	20021009	200336 B
US 20030073879	A1	20030417	US 2001328085	P	20011011	200340
			US 2002349270	P	20020118	
			US 2002268300	A	20021009	
US 6679827	B2	20040120	US 2001328085	P	20011011	200407
			US 2002349270	P	20020118	
			US 2002268300	A	20021009	

Priority Applications (No Type Date): US 2002349270 P 20020118; US 2001328085 P 20011011; US 2002268300 A 20021009
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 200330722	A2	E	13	A61B-000/00	

Designated States (National): AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
Designated States (Regional): AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW

US 20030073879	A1	A61N-002/00	Provisional application US 2001328085
			Provisional application US 2002349270
US 6679827	B2	A61N-002/04	Provisional application US 2001328085
			Provisional application US 2002349270

Treatment of tumor **involves creating elevated concentration of** free radicals **in** tumor 1, and creating magnetic field **that traverses** tumor **and inhibits recombination of** free radicals **in** tumor

Abstract (Basic):

... A **tumor** is treated by...

...a) creating an elevated concentration of **free radicals** in the **tumor** ; and...

...b) creating a **magnetic field** that traverses the **tumor** and that inhibits the recombination of the **free radicals** in the **tumor** .

... For treating a **tumor** .

...

...The inventive **method** creates an elevated concentration of **free radicals** in the **tumor** , and creates a **magnetic field** that traverses the **tumor** , inhibiting the recombination of the **free radicals** in the **tumor** , enhancing escape radical reactivity, resulting in enhanced **tumoricidal** effect...

...The figure shows an illustration of a **tumor** being treated by **radiation therapy** augmented by a **magnetic field** .

Technology Focus:

... Preferred Component: The **free radicals** are created in the **tumor** by means of electromagnetic radiation (12); by means of an introduction of a chemical agent; by sound waves; or by acoustic cavitations. The **free radical** reactivity is enhanced by, introducing electromagnetic shielding to block ambient **electromagnetic** interference. The **magnetic field** is created by **magnet** (s) (18) positioned exterior to the **tumor** ; or by magnetic particles injected into proximity to the **tumor** .

...

...Preferred Function: The **free radical** magnetic effects are contoured, scaled or designed to conform to **tumor** volume or shape...

...Preferred Condition: The electromagnetic radiation is in the frequency band of 1010-1020 Hz. The **magnetic field** is of a magnitude that facilitates the interstate crossing of singlet state **free radical** pairs to triplet state **free radical** pairs; has a magnitude of 0.1 Tesla to 10 milli Tesla through the **tumor** ; or it is of a magnitude that inhibits the interstate crossing of triplet state **free radical** pairs to singlet state **free radical** pairs...

....Preferred **Method** : The electromagnetic radiation is applied to the **tumor** in conjunction with the introduction of a chemical agent...

...Preferred Function: The **free radicals** interfere with the operation of enzymes within the **tumor** cells.

...Title Terms: **TUMOUR** ;

International Patent Class (Main): **A61B-000/00** ...

... **A61N-002/00** ...

... **A61N-002/04**

International Patent Class (Additional): **A61B-017/52**

19/3,K/5 (Item 5 from file: 350)
DIALOG(R)File 350:Derwent WPIX

013425201 **Image available**
WPI Acc No: 2000-597144/200057
XRPX Acc No: N00-442294
Measuring the electron spin resonance of a sample e.g. living body, involves using the electromagnetic **wave** magnetic field **component parallel to the surface of resonator loop**
Patent Assignee: YONESAWA DENSEN KK (YONE-N); ZH YAMAGATAKEN TECHNOPOLIS ZAIDAN (YAMA-N)
Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
JP 2000237165	A	20000905	JP 9946076	A	19990224	200057	B

Priority Applications (No Type Date): JP 9946076 A 19990224
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
JP 2000237165	A		5	A61B-005/055	

Measuring the electron spin resonance of a sample e.g. living body, involves using the electromagnetic **wave** magnetic field **component parallel to the surface of resonator loop**
Abstract (Basic):
... The electron spin resonance of a sample is measured using the **electromagnetic** wave **magnetic field** component parallel to the surface of resonator loop. This is done after impressing static and modulating **magnetic fields** in the direction perpendicular to the surface of the sample surface to which an electromagnetic...
... For detecting **free radicals** in living body vital to e.g. diagnosis, treatment, prophylaxis, of certain diseases e.g. **cancer** , inflammatory diseases, ulcers, cerebrovascular diseases, cardiac infraction...
International Patent Class (Main): **A61B-005/055**

19/3,K/6 (Item 6 from file: 350)
DIALOG(R)File 350:Derwent WPIX

013239937 **Image available**
WPI Acc No: 2000-411811/200035
XRAM Acc No: C00-124747

Drugs and diagnostics containing new or known N-acyloxy-substituted cyclic amine, for free radical scavenging, ESR, NMR, and treating conditions such as cancer , ischemia and neurodegeneration

Patent Assignee: DAIICHI RADIOISOTOPE LAB LTD (DARA); YAMAGATA TECHNOPOLIS FOUND (YAMA-N); YAMAGATA PUBLIC CORP DEV IND (YAMA-N)
Inventor: AOYAMA M; ITO O; OBARA H; YOKOYAMA H; ITOH O

Number of Countries: 009 Number of Patents: 005
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 200030638	A1	20000602	WO 99JP6523	A	19991122	200035	B
AU 200011863	A	20000613	AU 200011863	A	19991122	200043	
EP 1132085	A1	20010912	EP 99972535	A	19991122	200154	
			WO 99JP6523	A	19991122		
JP 2000583521	X	20020226	WO 99JP6523	A	19991122	200219	
			JP 2000583521	A	19991122		
US 6713492	B1	20040330	WO 99JP6523	A	19991122	200423	
			US 2001831898	A	20010524		

Priority Applications (No Type Date): JP 98334340 A 19981125
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 200030638	A1	J	37	A61K-031/40	
Designated States (National): AU CA JP US					
Designated States (Regional): DE FR GB IT NL					
AU 200011863	A			A61K-031/40	Based on patent WO 200030638
EP 1132085	A1	E		A61K-031/40	Based on patent WO 200030638
Designated States (Regional): DE FR GB IT NL					
JP 2000583521	X			A61K-031/40	Based on patent WO 200030638
US 6713492	B1			A01N-043/90	Based on patent WO 200030638

Drugs and diagnostics containing new or known N-acyloxy-substituted cyclic amine, for free radical scavenging, ESR, NMR, and treating conditions such as cancer , ischemia and neurodegeneration

Abstract (Basic):
... i) a **method** of scavenging active oxygen and **free radicals** in the body by treating with (I); and...

...I) hydrolyses in the body and forms the hydroxy amine, which reacts with **free radicals** to form nitroxide radicals that give ESR signals. 1-Acetoxy-3-carbamoyl-2,2,5...

...ESR-CT scan was performed at 720 MHz, 52 mW microwave and 25 mT core **magnetic field** , 15 mT band width, 0.2 mT magnetic variation, 100 kHz **magnetic field** frequency,. **magnetic** gradient 1 mT/cm, andgle 20 degrees. The hippocampus, cortex, corpus striatum, amygdala, and hypothalamus...

...I) and (II) are used for the prevention and treatment of ischemic conditions, digestive conditions, **cancer** , neurodegeneration associated with neurological disease, inflammation, glaucoma, and organ damage caused by drugs; and for...
...Title Terms: **CANCER** ;

```
Set     Items   Description
S1        124   AU=(SANDSTROM R? OR SANDSTROM, R?)
S2          0   ROBERT(2W)SANDSTROM
S3       2930   (MAGNET? OR ELECTROMAGNET? OR PARAMAGNET?) AND (TUMOR? OR -
            TUMOUR? OR CANCER? OR MALIGN? OR NEOPLAS? OR CARCIN?)
S4      17393   FREE()RADICAL?
S5     358299   IC=(A61B? OR A61N? OR A61M?)
S6         13   S1:S2 AND S3:S5
S7         13   IDPAT (sorted in duplicate/non-duplicate order)
? show files
File 347:JAPIO Nov 1976-2004/Feb(Updated 040607)
         (c) 2004 JPO & JAPIO
File 350:Derwent WPIX 1963-2004/UD,UM &UP=200437
         (c) 2004  Thomson Derwent
?
```

7/3,K/2 (Item 2 from file: 350)
DIALOG(R)File 350:Derwent WPIX

015320734 **Image available**
WPI Acc No: 2003-381669/200336
XRAM Acc No: C03-101420
XRPX Acc No: N03-304865
Treatment of tumor **involves creating elevated concentration of** free radicals **in** tumor , **and creating** magnetic **field that traverses** tumor **and inhibits recombination of** free radicals **in** tumor
Patent Assignee: SANDSTROM R E (SAND-I)
Inventor: **SANDSTROM R E**
Number of Countries: 097 Number of Patents: 003
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
WO 200330722	A2	20030417	WO 2002US32444	A	20021009	200336 B
US 20030073879	A1	20030417	US 2001328085	P	20011011	200340
			US 2002349270	P	20020118	
			US 2002268300	A	20021009	
US 6679827	B2	20040120	US 2001328085	P	20011011	200407
			US 2002349270	P	20020118	
			US 2002268300	A	20021009	

Priority Applications (No Type Date): US 2002349270 P 20020118; US 2001328085 P 20011011; US 2002268300 A 20021009
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 200330722	A2	E	13	A61B-000/00	
Designated States (National): AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW				
Designated States (Regional): AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW					
US 20030073879	A1			A61N-002/00	Provisional application US 2001328085
					Provisional application US 2002349270
US 6679827	B2			A61N-002/04	Provisional application US 2001328085
					Provisional application US 2002349270

Treatment of tumor **involves creating elevated concentration of** free radicals **in** tumor 1, and creating magnetic **field that traverses** tumor **and inhibits recombination of** free radicals **in** tumor
Inventor: **SANDSTROM R E**

Abstract (Basic):
... A **tumor** is treated by...
...a) creating an elevated concentration of **free radicals** in the **tumor** ; and...

...b) creating a **magnetic** field that traverses the **tumor** and that inhibits the recombination of the **free radicals** in the **tumor** .
... For treating a **tumor** .
...

...The inventive method creates an elevated concentration of **free radicals** in the **tumor** , and creates a **magnetic** field that traverses the **tumor** , inhibiting the recombination of the **free radicals** in the **tumor** , enhancing escape radical reactivity, resulting in enhanced **tumoricidal** effect...

...The figure shows an illustration of a **tumor** being treated by radiation therapy augmented by a **magnetic** field...

... **Electromagnetic** radiation (12...

... **Magnet** (18
Technology Focus:
... Preferred Component: The **free radicals** are created in the **tumor** by means of **electromagnetic** radiation (12); by means of an introduction of a chemical agent; by sound waves; or by acoustic cavitations. The **free radical** reactivity is enhanced by, introducing **electromagnetic** shielding to block ambient **electromagnetic** interference. The **magnetic** field is created by **magnet** (s) (18) positioned exterior to the **tumor** ; or by **magnetic** particles injected into proximity to the **tumor** .
...

...Preferred Function: The **free radical magnetic** effects are contoured, scaled or designed to conform to **tumor** volume or shape...

...Preferred Condition: The **electromagnetic** radiation is in the frequency band of 1010-1020 Hz. The **magnetic** field is of a magnitude that facilitates the interstate crossing of singlet state **free radical** pairs to triplet state **free radical** pairs; has a magnitude of 0.1 Tesla to 10 milli Tesla through the **tumor** ; or it is of a magnitude that inhibits the interstate crossing of triplet state **free radical** pairs to singlet state **free radical** pairs...

...Preferred Method: The **electromagnetic** radiation is applied to the **tumor** in conjunction with the introduction of a chemical agent...
...Preferred Function: The **free radicals** interfere with the operation of enzymes within the **tumor** cells.
...Title Terms: **TUMOUR** ;
International Patent Class (Main): **A61B-000/00** ...

... **A61N-002/00** ...

... **A61N-002/04**
International Patent Class (Additional): **A61B-017/52**

```
Set     Items   Description
S1        130   AU=(SANDSTROM R? OR SANDSTROM, R?)
S2          0   ROBERT(2W)SANDSTROM
S3      10289   (MAGNET? OR ELECTROMAGNET? OR PARAMAGNET?) AND (TUMOR? OR -
             TUMOUR? OR CANCER? OR MALIGN? OR NEOPLAS? OR CARCIN?)
S4      30484   FREE()RADICAL?
S5      94371   IC=(A61B? OR A61N? OR A61M?)
S6          7   S1:S2 AND S3:S5
S7          7   IDPAT (sorted in duplicate/non-duplicate order)
? show files
File 348:EUROPEAN PATENTS 1978-2004/Jun W02
         (c) 2004 European Patent Office
File 349:PCT FULLTEXT 1979-2002/UB=20040610,UT=20040603
         (c) 2004 WIPO/Univentio
```

7/3,AU/1 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01591782
MAGNETIC FIELD ENHANCEMENT OF TUMOR TREATMENT
AMELIORATION D'UN TRAITEMENT TUMORAL AU MOYEN D'UN CHAMP MAGNETIQUE
PATENT ASSIGNEE:
Sandstrom, Robert E., (4408930), 49 View Ridge Lane, Longview, WA 98632, (US), (Applicant designated States: all)
INVENTOR:
Sandstrom, Robert E. , 49 View Ridge Lane, Longview, WA 98632, (US
PATENT (CC, No, Kind, Date):
WO 2003030722 030417
APPLICATION (CC, No, Date): EP 2002780439 021009; WO 2002US32444 021009
PRIORITY (CC, No, Date): US 328085 P 011011; US 349270 P 020118
DESIGNATED STATES: AT; BE; BG; CH; CY; CZ; DE; DK; EE; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT
EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI
INTERNATIONAL PATENT CLASS: **A61B-001/00**
LANGUAGE (Publication,Procedural,Application): English; English; English

7/3,AU/2 (Item 2 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

01001137
MAGNETIC FIELD ENHANCEMENT OF TUMOR **TREATMENT**
AMELIORATION D'UN TRAITEMENT TUMORAL **AU MOYEN D'UN CHAMP MAGNETIQUE**
Patent Applicant/Inventor:
Sandstrom Robert E , 49 View Ridge Lane, Longview, WA 98632, US, US (Residence), US (Nationality
Legal Representative:
Siegel Timothy E (agent), 1868 Knapps Alley,, Suite 206, West Linn, OR 97068-4644, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200330722 A2-A3 20030417 (WO 0330722)
Application: WO 2002US32444 20021009 (PCT/WO US0232444)
Priority Application: US 2001328085 20011011; US 2002349270 20020118
Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LU MC NL PT SE SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 2309

```
Set     Items   Description
S1        946   AU=(SANDSTROM R? OR SANDSTROM, R?)
S2          0   ROBERT(2W)SANDSTROM
S3     201333   (MAGNET? OR ELECTROMAGNET? OR PARAMAGNET?) AND (TUMOR? OR -
            TUMOUR? OR CANCER? OR MALIGN? OR NEOPLAS? OR CARCIN?)
S4     298485   FREE()RADICAL?
S5          1   S1:S2 AND S3
S6          1   S1:S2 AND S4
S7          1   S5:S6
? show files
File 155:MEDLINE(R) 1966-2004/Jun W1
         (c) format only 2004 The Dialog Corp.
File   2:INSPEC 1969-2004/Jun W1
         (c) 2004 Institution of Electrical Engineers
File   5:Biosis Previews(R) 1969-2004/Jun W1
         (c) 2004 BIOSIS
File   6:NTIS 1964-2004/Jun W2
         (c) 2004 NTIS, Intl Cpyrght All Rights Res
File   8:Ei Compendex(R) 1970-2004/Jun W1
         (c) 2004 Elsevier Eng.  Info. Inc.
File  34:SciSearch(R) Cited Ref Sci 1990-2004/Jun W1
         (c) 2004 Inst for Sci Info
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
         (c) 1998 Inst for Sci Info
File  73:EMBASE 1974-2004/Jun W1
         (c) 2004 Elsevier Science B.V.
File  71:ELSEVIER BIOBASE 1994-2004/Jun W1
         (c) 2004  Elsevier Science B.V.
File 144:Pascal 1973-2004/Jun W1
         (c) 2004 INIST/CNRS
File  35:Dissertation Abs Online 1861-2004/May
         (c) 2004 ProQuest Info&Learning
File  65:Inside Conferences 1993-2004/Jun W2
         (c) 2004 BLDSC all rts. reserv.
File  94:JICST-EPlus 1985-2004/May W4
         (c)2004 Japan Science and Tech Corp(JST)
File  95:TEME-Technology & Management 1989-2004/May W4
         (c) 2004 FIZ TECHNIK
File  99:Wilson Appl. Sci & Tech Abs 1983-2004/May
         (c) 2004 The HW Wilson Co.
File 481:DELPHES Eur Bus 95-2004/May W5
         (c) 2004 ACFCI & Chambre CommInd Paris
File 583:Gale Group Globalbase(TM) 1986-2002/Dec 13
         (c) 2002 The Gale Group
?
```

7/3,K/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0014733801 BIOSIS NO.: 200400104558
Magnetic **field enhancement of** tumor **treatment**
AUTHOR: **Sandstrom Robert E** (Reprint
AUTHOR ADDRESS: 49 View Ridge La., Longview, WA, 98632, USA**USA
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents 1278 (3): Jan. 20, 2004 2004
MEDIUM: e-file
PATENT NUMBER: US 6679827 PATENT DATE GRANTED: January 20, 2004 20040120
PATENT CLASSIFICATION: 600-9 PATENT COUNTRY: USA
ISSN: 0098-1133 (ISSN print)
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

Magnetic **field enhancement of** tumor **treatment**
AUTHOR: **Sandstrom Robert E** ...

ABSTRACT: A method of creating an elevated concentration of **free radicals** having augmented lifetimes within a **tumor** , that includes creating an elevated concentration of **free radicals** in the **tumor** and creating a **magnetic** field that traverses the **tumor** and that inhibits the recombination of the **free radicals** in the **tumor** . A **magnetic** field of 0.1 mTesla to 10 mTesla is generally used for this purpose.
DESCRIPTORS:
DISEASES: tumors --...

... **neoplastic** disease, therapy
MESH TERMS: **Neoplasms** (MeSH)
METHODS & EQUIPMENT: **magnetic** field **tumor** therapy enhancement...

```
Set     Items   Description
S1         14   AU=(SANDSTROM R? OR SANDSTROM, R?)
S2          6   ROBERT(2W)SANDSTROM
S3      17908   (MAGNET? OR ELECTROMAGNET? OR PARAMAGNET?) AND (TUMOR? OR -
             TUMOUR? OR CANCER? OR MALIGN? OR NEOPLAS? OR CARCIN?)
S4      16414   FREE()RADICAL?
S5          0   S1:S2 AND S3:S4
? show files
File  16:Gale Group PROMT(R) 1990-2004/Jun 15
         (c) 2004 The Gale Group
File 160:Gale Group PROMT(R) 1972-1989
         (c) 1999 The Gale Group
File 148:Gale Group Trade & Industry DB 1976-2004/Jun 15
         (c)2004 The Gale Group
File 149:TGG Health&Wellness DB(SM) 1976-2004/Jun W1
         (c) 2004 The Gale Group
File 621:Gale Group New Prod.Annou.(R) 1985-2004/Jun 15
         (c) 2004 The Gale Group
File 444:New England Journal of Med. 1985-2004/Jun W2
         (c) 2004 Mass. Med. Soc.
File 441:ESPICOM Pharm&Med DEVICE NEWS 2004/Jun W2
         (c) 2004 ESPICOM Bus.Intell.
File 369:New Scientist 1994-2004/Jun W1
         (c) 2004 Reed Business Information Ltd.
File 370:Science 1996-1999/Jul W3
         (c) 1999 AAAS
File 129:PHIND(Archival) 1980-2004/Jun W1
         (c) 2004 PJB Publications, Ltd.
File 130:PHIND(Daily & Current) 2004/Jun 14
         (c) 2004 PJB Publications,Ltd.
File 135:NewsRx Weekly Reports 1995-2004/Jun W1
         (c) 2004 NewsRx
File  98:General Sci Abs/Full-Text 1984-2004/Jun
         (c) 2004 The HW Wilson Co.
File  15:ABI/Inform(R) 1971-2004/Jun 14
         (c) 2004 ProQuest Info&Learning
?
```

US005437600A

United States Patent [19]

Liboff et al.

[11] Patent Number: 5,437,600

[45] Date of Patent: Aug. 1, 1995

[54] METHOD AND APPARATUS FOR THE TREATMENT OF CANCER

[75] Inventors: **Abraham R. Liboff**, Birmingham, Mich.; **Bruce R. McLeod**, Bozeman, Mont.; **Stephen D. Smith**, Lexington, Ky.

[73] Assignee: **Life Resonances, Inc.**, Bozeman, Mont.

[21] Appl. No.: **268,061**

[22] Filed: **Jun. 27, 1994**

Related U.S. Application Data

[63] Continuation of Ser. No. 3,787, Jan. 13, 1993, abandoned, which is a continuation of Ser. No. 902,929, Jun. 23, 1992, Pat. No. 5,183,456, which is a continuation of Ser. No. 703,383, May 21, 1991, Pat. No. 5,211,622, which is a continuation of Ser. No. 437,485, Nov. 15, 1989, Pat. No. 5,045,050.

[51] **Int. Cl.**[6] **A61N 2/04**

[52] **U.S. Cl.** **600/9**

[58] **Field of Search** 607/100, 103; 128/653.1; 600/9, 13, 15

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,105,017	3/1978	Ryaby et al.	128/1.5
4,134,395	1/1979	Davis	600/9
4,317,078	2/1982	Weed et al.	128/653.1
4,428,366	1/1984	Findl et al.	128/15
4,528,265	7/1985	Becker	128/419 F
4,548,208	10/1985	Niemi	128/419
4,561,426	12/1985	Stewart	128/1.5
4,587,957	5/1986	Castel	600/9
4,622,952	11/1986	Gordon	128/1.3
4,654,574	1/1987	Thaler	320/14
4,665,898	5/1987	Costa et al.	600/14
4,683,873	3/1987	Cadossi et al.	128/1.5
4,818,697	1/1989	Liboff et al.	435/173
4,932,951	6/1990	Liboff et al.	606/13
5,045,050	9/1991	Liboff et al.	600/9
5,087,438	2/1992	Gordon	600/9
5,106,361	4/1992	Liboff et al.	600/15
5,108,359	4/1992	Granov et al.	600/9
5,123,898	6/1992	Liboff et al.	128/419 F

FOREIGN PATENT DOCUMENTS

38255/85	8/1985	Australia .
1113156	11/1981	Canada .

OTHER PUBLICATIONS

"Geomagnetic Cyclotron Resonance in Living Cells" Liboff, A. R., Journal of Biologicl Physics, vol. 13, 1985.

"Cyclotron Resonance in Membrane Transport" Liboff, A. R., *Interactions Between Electromagnetic Fields and Cells*, 1985.

"A Rôle for the Magnetic Field in the Radiation-Induced Efflux of Calcium Ions from Brain Tissue In Vitro" Blackman, C. F., et al. Bioelectromagnetics 6:327-337 (1985).

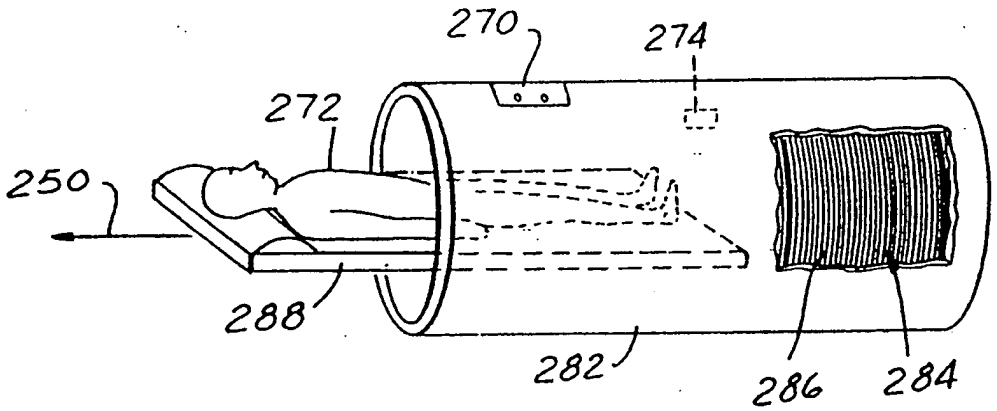
Primary Examiner—George Manuel

Attorney, Agent, or Firm—Dykema Gossett

[57] **ABSTRACT**

A method and apparatus for therapeutically treating cancer is provided. The apparatus includes a magnetic field generator for producing a controlled, fluctuating, directionally oriented magnetic field parallel to a predetermined axis projecting through a malignant neoplasm. In one aspect, a field detector measures the magnetic flux density along the predetermined axis. The applied magnetic field may comprise a full-wave rectified signal oscillated at predetermined frequencies to maintain a preselected ratio of frequency to the effective flux density, where the ratio regulates the growth characteristics of cancer cells of the neoplasm. This ratio is maintained by adjusting the frequency of the fluctuating magnetic field and/or by adjusting the intensity of the applied magnetic field after nulling out the local magnetic field at that region containing the neoplasm. In one aspect, a synergistic therapeutic cancer treatment is obtained by exposing cancer cells to the magnetic fields of the invention in the presence of a chemotherapeutic cancer agent.

2 Claims, 3 Drawing Sheets



US005156587A

United States Patent [19]

Montone

[11] Patent Number: 5,156,587

[45] Date of Patent: Oct. 20, 1992

[54] METHOD FOR TREATING MALIGNANT CELLS

[76] Inventor: Liber J. Montone, 9242 Vanderbilt Dr., Naples, Fla. 33963

[21] Appl. No.: 3,782

[22] Filed: Jan. 9, 1987

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 528,442, Sep. 1, 1983, abandoned.

[51] Int. Cl.[5] A61N 2/04

[52] U.S. Cl. 600/13

[58] Field of Search 128/1.3, 1.5; 600/9, 600/13, 15

[56] References Cited

FOREIGN PATENT DOCUMENTS

506966 8/1978 U.S.S.R. 128/1.5

1595108 8/1981 United Kingdom 128/1.5

OTHER PUBLICATIONS

Lenzi, Radiology, Sep. 1940, pp. 307-314.

Barnothy, Medical Physics, vol. 3, 1960, pp. 61-62.

Mansfield et al., NMR Imaging in Biomedicine, Sup. 2, Academic Press, N.Y., 1982.

Harrison's Principles of Internal Medicine, 10th Ed., McGraw Hill, N.Y., 1983, pp. 834 and 1761-1762.

Cramp, I-ON-A CO—The Magic Horse Collar, reprint from Gygeia, Feb. 1927.

Solov'eva et al., Biomdeical Engr., vol. 7, No. 5, p. 2914, Sep.-Oct., 1973.

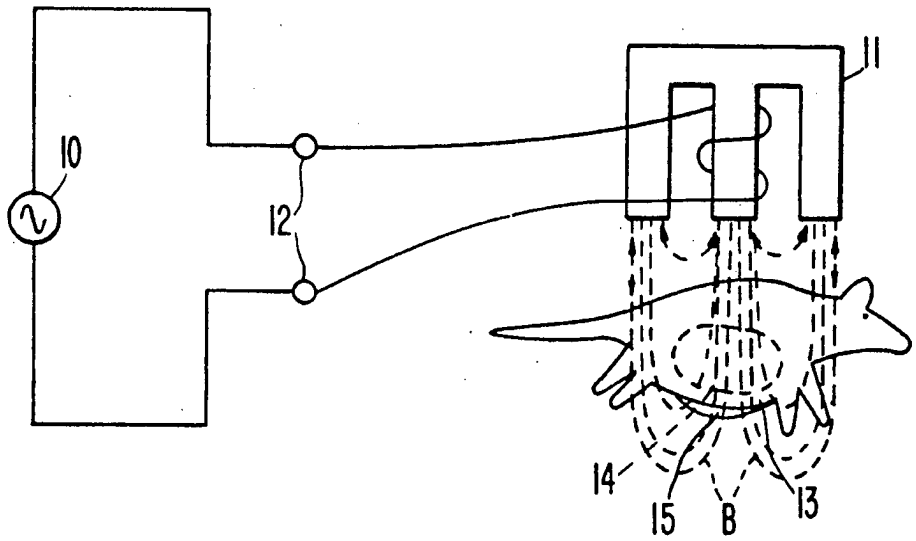
Primary Examiner—Lee S. Cohen

Attorney, Agent, or Firm—Gregory E. Montone

[57] ABSTRACT

A method and apparatus for destroying or retarding growth of malignant cells and tumors using one or more coils of wire, externally applied to the body, for chosen periods, which are connected to an alternating current source to produce a low frequency sinusoidal magnetic field of desired intensity at the irradiated malignant region to be treated.

21 Claims, 4 Drawing Sheets



US005099756A

United States Patent [19]

Franconi et al.

[11] Patent Number: 5,099,756

[45] Date of Patent: Mar. 31, 1992

[54] **RADIO FREQUENCY THERMOTHERAPY**

[75] Inventors: Cafiero Franconi; Carlo A. Tiberio, both of Rome, Italy; Harry H. LeVeen, 321 Confederate Cir., Charleston, S.C. 29407

[73] Assignee: Harry H. LeVeen, Charleston, S.C.

[21] Appl. No.: 360,244

[22] Filed: Jun. 1, 1989

[51] Int. Cl.[5] A61N 2/04

[52] U.S. Cl. 600/10; 600/13; 600/14; 128/804; 336/229; 219/10.79

[58] Field of Search 600/9, 10, 12, 13, 14; 128/804; 336/229; 219/10.79

[56] References Cited

U.S. PATENT DOCUMENTS

3,890,953	6/1975	Kraus et al.	600/14
4,325,361	4/1982	Harrison	600/10
4,402,309	9/1983	Harrison	600/10
4,674,481	6/1987	Boddie, Jr. et al.	600/10

FOREIGN PATENT DOCUMENTS

3333288	4/1985	Fed. Rep. of Germany	600/13
3721864	1/1989	Fed. Rep. of Germany	600/13
1007681	3/1983	U.S.S.R.	600/13

OTHER PUBLICATIONS

Y. Kotsuka et al., "Ferrite Applicator and Implant Material for Local Hyperthermia of Induction Heating", in: T. Sugahara and M. Saito (eds.), Proc. Symp. on Hyperthermic Oncology, vol. 1, pp. 843-844, Taylor and Francis, Bristol, 1989.

Y. Saitoh et al., "A Re-Entrant Resonant Cavity Applicator for Deep and Concentrated Hyperthermia", in: T. Sugahara and M. Saito (eds.), Proc. Symp. on Hyperthermic Oncology, vol. 1, pp. 837-838, Taylor and Francis, Bristol, 1989.

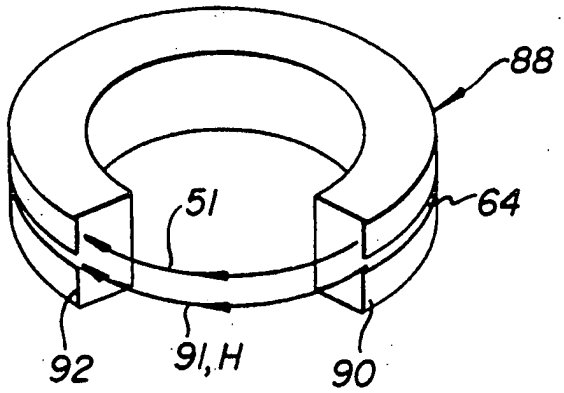
Primary Examiner—Lee S. Cohen

Attorney, Agent, or Firm—Gipple & Hale

[57] **ABSTRACT**

Apparatus for treating neoplasms in humans and animals comprising a hollow toroidal applicator which resonates at a specific radiofrequency, and which possess electrically conductive walls on which radial radiofrequency currents flow and generate a high density of uninterrupted magnetic flux within the hollow body. A rotatable antenna connected to a source of radiofrequency power is mounted inside the applicator body to couple with the electromagnetic field of the applicator. The body part to be treated is interposed through side apertures or through the space created by removing a segment of the toroid which can have orifices of predetermined cross sectional areas across which a tubular zone of high magnetic flux travels through the interposed tumor and normal tissue to induce more heat in the interposed tumor tissue than in the interposed normal tissue.

15 Claims, 12 Drawing Sheets



United States Patent [19]

Costa et al.

[11] **Patent Number: 4,665,898**

[45] **Date of Patent: May 19, 1987**

[54] **MALIGNANCY TREATMENT**

[75] Inventors: **Jonathan L. Costa**, Bethesda, Md.; **Gunter A. Hofmann**, San Diego, Calif.

[73] Assignee: **Maxwell Laboratories, Inc.**, San Diego, Calif.

[21] Appl. No.: **613,507**

[22] Filed: **May 23, 1984**

[51] **Int. Cl.**[4] **A61B 17/52**

[52] **U.S. Cl.** **128/1.3**

[58] **Field of Search** 128/1.3-1.5, 128/804; 422/22; 426/234, 237, 238, 241

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,368,155	2/1968	Priore	128/1.3
3,467,076	9/1969	Frisch et al.	128/1.3
4,134,395	1/1979	Davis	128/1.3
4,323,056	4/1982	Borrelli et al.	128/1.3
4,510,925	4/1985	Constantinescu	128/1.3
4,524,079	6/1985	Hofmann	426/234

FOREIGN PATENT DOCUMENTS

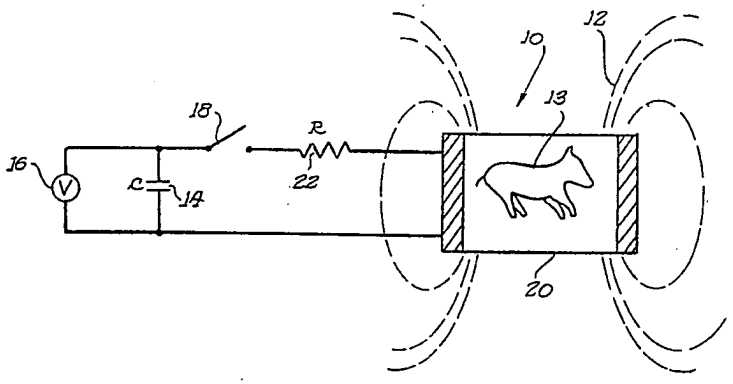
0040053	11/1981	European Pat. Off.	128/1.3
0039988	11/1981	European Pat. Off.	128/1.3
2253686	11/1974	Fed. Rep. of Germany .	
2370483	7/1978	France	128/1.3
1416335	12/1975	United Kingdom	128/1.3

Primary Examiner—Edward M. Coven
Assistant Examiner—Max F. Hindenburg
Attorney, Agent, or Firm—Fitch, Even, Tabin & Flannery

[57] **ABSTRACT**

A body part of an animal afflicted with malignant cells is disposed within a magnetic coil and subjected to a plurality of magnetic field pulses, the pulses having intensities of between about 1 and about 100 Tesla and characteristic frequencies of between about 5 and about 1000 kHz. The pulsed magnetic field selectively inactivates and/or destroys malignant cells with relatively little damage to normal tissue as compared to conventional radiation therapy procedures.

15 Claims, 1 Drawing Figure



```
Set     Items   Description
S1      24335   (MAGNET? OR ELECTROMAGNET? OR PARAMAGNET? OR FERROMAGNET?)-
             (3N)(FIELD? OR SPHERE? OR AREA? OR REGION? OR ZONE? OR PENUMB-
             R?)
S2       9873   MRI OR MAGNET?()RESONAN?
S3     116673   CANCER? OR TUMOR? OR TUMOUR? OR MALIGNAN? OR NEOPLAS? OR N-
             EO()PLAS? OR CARCINO?
S4      32407   FREE()RADICAL? OR OXYGEN()RADICAL?
S5        277   ATOM?(5N)(UNPAIR? OR UNMATCH? OR LOST)(2N)ELECTRON? ?
S6      37351   APOPTO? OR NECROSIS? OR NECROTI? OR NECROBIO? OR TUMORCID?
             OR TUMOURCID?
S7      31663   (CELL? ? OR CELLULAR?)(2N)(DEATH? OR DIE OR DIES OR DIED OR
              DYING OR KILL? OR SUICID?)
S8      18513   (THERAPY? OR THERAPEUT? OR THERAPIE? OR TREATMENT?)(3N)(RA-
             DIAT? OR GAMMA? OR GAMMARAY? OR SOUND? OR ACOUSTIC? OR ULTRAS-
             OUND? OR ULTRASON? OR ULTRA()(SONIC? OR SOUND?) OR PHOTO? OR -
             XRAY? OR X()(RAY OR RAYS OR RAYING OR RAYED))
S9    1323268   METHOD? ?
S10   1155122   SYSTEM? ?
S11   1026429   PROCESS??
S12    451222   PROCEDURE? ?
S13    581669   TECHNIQUE? ?
S14     94371   IC=(A61B? OR A61N? OR A61M?)
S15       187   S1 AND S3 AND S4:S5
S16        78   S15 AND S6:S7
S17        47   S15:S16 AND S6:S7(5N)S3
S18        25   S17 AND S8
S19        47   S17 AND S9:S13
S20        16   S17 AND S14
S21       187   S15:S16
S22       143   S21 NOT S2
S23        60   S22 AND S4:S5 AND S6:S7
S24        74   S17:S20 OR S23
S25        74   IDPAT (sorted in duplicate/non-duplicate order)
? show files
File 348:EUROPEAN PATENTS 1978-2004/Jun W02
         (c) 2004 European Patent Office
File 349:PCT FULLTEXT 1979-2002/UB=20040610,UT=20040603
         (c) 2004 WIPO/Univentio
?
```

Display Abstract Show: 20 Sort Send to Text

1: Photochem Photobiol. 1994 Jun;59(6):585-9.

Related Articles, Links

Model for the rationalization of magnetic field effects in vivo. Application of the radical pair mechanism to biological systems.

Scaiano JC, Cozens FL, McLean J.

Department of Chemistry, University of Ottawa, Canada.

A model for magnetic field effects in biological systems is proposed. This model employs the basic concepts of the radical pair mechanism, and predicts that magnetic fields will increase the average radical concentration, lengthen their lifetime and enhance the probability of radical reactions with cellular components. The relevance of these effects in relation to cancer initiation, promotion and progression is discussed.

Publication Types:
- Review
- Review, Tutorial

PMID: 8066117 [PubMed - indexed for MEDLINE]

Display Abstract Show: 20 Sort Send to Text

Jun 7 2004 18:11:57

1: J Nucl Med. 1994 Jan;35(1):157-63.

Magnetically enhanced radionuclide therapy.

Raylman RR, Wahl RL.

Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI 48109-0552.

Radiopharmaceutical therapy is an increasingly common treatment for cancer. This therapy involves the injection of radiolabeled tumor-specific agents into the patient with subsequent preferential accumulation in the tumor sites. Particulate radiation (usually beta particles) emitted by the radioisotope kill or damage the tumor cells. The effectiveness of radiopharmaceutical therapy, however, is limited by the size of the tumor treated. Energetic particles can easily exist small tumors before they are able to deposit their energy and inflict significant damage. METHODS: We propose the use of a static magnetic field to be applied after the radiopharmaceutical has localized in the tumors, constraining these particles to helical paths. This application would result in substantially confining the emitted particles within the tumor's boundaries, thus increasing radiation dose to the tumor. RESULTS: Computer simulations of radionuclide treatments using 131I, 186Re and 90Y show that a magnetic field of 10 Tesla can increase the radiation dose achieved by conventional radionuclide therapy by up to 71%. In addition, total radiation dose to surrounding normal tissues is substantially reduced. CONCLUSION: Magnetically enhanced radionuclide therapy (MERiT) therefore shows promise as an effective treatment of cancer and warrants further study.

PMID: 8271038 [PubMed - indexed for MEDLINE]

Display Abstract Show: 20 Sort Send to Text

Jun 7 2004 18:11:57

1: Int J Radiat Oncol Biol Phys. 1997 Mar 15;37(5):1201-6.

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Magnetically-enhanced radionuclide therapy (MERiT): in vitro evaluation.

Raylman RR, Clavo AC, Crawford SC, Recker B, Wahl RL.

Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, USA.
rraylman@wvuhsc1.hsc.wvu.edu

PURPOSE: Radionuclide therapy is a promising method for delivering radiation dose selectively to tumors. In situations where electron -emitters are used and the tumor is small relative to the maximum range of therapeutic electrons, these particles exit the tumor before delivering the maximum amounts of radiation dose. In this study, the method of magnetically constraining electrons to small tumors, known as magnetically -enhanced radionuclide therapy (MERiT), is explored using in vitro experiments. METHODS AND MATERIALS: The potential utility of MERiT was investigated by first measuring the reduction of number of electrons exiting a small sphere containing 90Y embedded in a block of plastic scintillator. Measurements of total energy deposited in the plastic scintillator made inside and outside a 7 Tesla magnetic field were compared. Furthermore, an experiment utilizing lymphoma cells of human origin was performed. Groups of cells were added to wells containing 90Y-labeled bovine serum albumin (and control groups containing no radioactivity) were placed either inside a 7 Tesla magnet or at a position where the magnetic field was minimal (essentially zero) for 18 hr. RESULTS: The presence of a 7 Tesla magnetic field reduced the amount of energy deposited in the scintillator by 16.63 +/- 1.05%. This demonstrates that the magnetic field constrains a large fraction of the emissions to the sphere and implies that normal tissues adjacent to radiotracer-avid tumors can be protected from radiation dose. Results from the cell culture experiment showed that the presence of a 7 Tesla magnetic field significantly ($p < 0.005$) reduced the number of viable cells remaining after treatment with non-specific 90Y-labeled bovine serum albumin by 11.7% compared to the appropriate control group (90Y treated, not exposed to magnetic field).

CONCLUSIONS: These initial physical and biological studies indicate that magnetically-enhanced radionuclide therapy can be effective in increasing radiation absorbed dose to small tumors, consequently reducing radiation dose to surrounding normal structures.

PMID: 9169832 [PubMed - indexed for MEDLINE]

The International Journal of Biochemistry & Cell Biology
Volume 32, Issue 2 , February 2000, Pages 157-170

doi:10.1016/S1357-2725(99)00088-6 Cite or Link Using DOI

Review

Role of reactive oxygen species in apoptosis: implications for cancer therapy

José M. Matés and **Francisca M. Sánchez-Jiménez**

Department of Molecular Biology and Biochemistry, Sciences Faculty, University of Málaga, Campus de Teatinos, s/n, 29071 Málaga, Spain

Received 6 January 1999; accepted 2 July 1999. Available online 17 December 1999.

Abstract

Reactive oxygen species are widely generated in biological systems. Consequently humans have evolved antioxidant defence systems that limit their production. Intracellular production of active oxygen species such as –.OH, O_2 and H_2O_2 is associated with the arrest of cell proliferation. Similarly, generation of oxidative stress in response to various external stimuli has been implicated in the activation of transcription factors and to the triggering of apoptosis. Here we review how free radicals induce DNA sequence changes in the form of

mutations, deletions, gene amplification and rearrangements. These alterations may result in the initiation of apoptosis signalling leading to cell death, or to the activation of several proto-oncogenes and/or the inactivation of some tumour suppressor genes. The regulation of gene expression by means of oxidants, antioxidants and the redox state remains as a promising therapeutic approach. Several anticarcinogenic agents have been shown to inhibit reactive oxygen species production and oxidative DNA damage, inhibiting tumour promotion. In addition, recombinant vectors expressing radical-scavenging enzymes reduce apoptosis. In conclusion, oxidative stress has been implicated in both apoptosis and the pathogenesis of cancer providing contrived support for two notions: free radical reactions may be increased in malignant cells and oxidant scavenging systems may be useful in cancer therapy.

Author Keywords: Antioxidant enzymes; Apoptosis; Cancer; Oxidative damage; Reactive oxygen species

Abbreviations: ALL, acute lymphoblastic leukaemia; AP-1, activated protein-1; ASK1, apoptosis signal-regulating kinase 1; c-AMP, adenosine cyclic monophosphate; CAT, catalase; CLL, chronic lymphatic leukaemia; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; GPX, glutathione peroxidase; GSH, glutathione; LDLs, low-density lipoproteins; LPS, lipopolysaccharide; MAPKs, mitogen-activated protein kinases; MPO, mycloperoxidase; $NF_{\kappa}B$, nuclear transcription factor kappa B; oxLDL, oxidized low-density lipoproteins; ROS, reactive oxygen species; SOD, superoxide dismutase; TGFβ, transforming growth factor beta; TNF, tumour necrosis factor

---

Article Outline

1. Introduction

As a consequence of aerobic metabolism small amounts of reactive oxygen species (ROS), including hydroxyl radicals (.OH), superoxide anions ($O_2^{\cdot}$), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2), are constantly generated in organisms [1]. Cellular antioxidants act in concert to detoxify these species but, when the balance is disrupted, a condition referred to as oxidative stress exists. If oxidative stress persists, oxidative damage to critical biomolecules (including oxidant-induced damage to the genome) accumulates and eventually results in several biological effects ranging from alterations in signal transduction and gene expression to mitogenesis, transformation, mutagenesis and cell death [2 and 3].

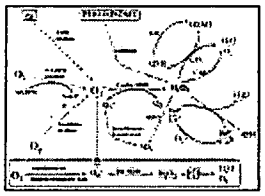
Apoptosis and cancer are opposed phenomena, but ROS have been widely reported to play a key role in both. Evidences that apoptosis can be induced by ROS is provided by studies in which mediators of apoptosis, induce intracellular production of ROS or are inhibited by the addition of antioxidants. Although the mechanism involved is still controversial redox status and/or hydrogen peroxide have both been proposed as critical factors [4 and 5]. In addition, induction of carcinogenesis has been clearly linked to oxidative DNA damage [3] and the DNA oxidative product, 8-oxo-2′-deoxyguanosine, has been reported to be highly mutagenic [6]. ROS are thought to contribute to carcinogenesis through interference with signal cascade systems, including among others, the nuclear transcription factor kappa B ($NF_{\kappa}B$), activated protein-1 (AP-1), phospholipase A_2, mitogen-activated protein kinases (MAPKs) and c-Jun kinase [7, 8, 9 and 10].

Cells react rapidly to redox imbalance with a plethora of biological responses, including cell cycle-specific growth arrest, gene transcription, initiation of signal transduction pathways and repair of damaged DNA. These early events are likely to determine whether a cell will necrose, senesce, apoptose or survive and proliferate [11].

Many tumours have been associated with inhibition of apoptosis, follicular lymphomas, carcinomas with p53 mutations: medullary breast carcinoma, lung cancer, colorectal cancer; and hormone-dependent tumours: such as breast, prostate and ovarian cancer [12, 13 and 14].

2. Source and control of ROS production

In aerobic cells, the most important sources of $O_2^{\cdot}$ are the electron transport chains of mitochondria and endoplasmic reticulum. In mitochondria, ROS formation is significantly increased by uncouplers of oxidative phosphorylation, hyperbaric O_2 treatment, pathologic conditions such as ischemia/reperfusion syndrome, ageing, etc and alterations of mitochondrial lipids occurring during deficiency of polyunsaturated fatty acids and lipoperoxidation processes. In the endoplasmic reticulum (RE) NADPH-cytochrome P450 reductase can leak electrons onto O_2 generating $O_2^{\cdot}$ (Fig. 1). It can also be formed during activity of the desaturase system, which introduce C–C double bonds into unsaturated fatty acids. The system contains $FADH_2$ and cytochrome b_5 that can leak electrons onto O_2 [15].



(19K)

Fig. 1. Generation of reactive oxygen species and main defence mechanisms against damage produced by reactive oxygen species. During hypoxia, generated superoxide may be degraded into the mitochondria by Mn-SOD or by Cu, Zn-SOD. Other sources of ROS and enzymes implicated in detoxification are shown in the figure and discussed in the text.

Other biological sources of $O_2^{\cdot}$ are the nuclear membrane (containing an electron transport chain that in the presence of NAD(P)H is able to leak electrons onto O_2 with formation of $O_2^{\cdot}$), the decomposition of oxyhaemoglobin, photo-irradiation of tryptophan, eumelanin and pheomelanin by UV-light, endothelium, autoxidation of catecholamines, thiols, a reduced form of riboflavin and its derivatives, enzymes such as xanthine oxidase, dyoxygenases and oxidases and phagocyte cells as neutrophils and macrophages [16].

Generation of hydrogen peroxide takes place through the dismutation of superoxide.

Therefore any biological system generating O_2. will produce H_2O_2. However, there are enzymes localized in peroxisomes that produce H_2O_2 without intermediation of O_2. . Contrary to O_2. , H_2O_2 is able to cross cell membranes and inside the cells it can react with Fe^{2+} or Cu^{+} to form hydroxyl radicals via Fenton reaction [17 and 18].

$$Fe^{2+}+H_2O_{\rightarrow}Fe^{3+}+.OH+\ \ OH \text{ (Fenton reaction)}$$

$$O_2.\ +H_2O_{2\rightarrow}O_2+.OH^{+}\ \ OH \text{ (Haber–Weiss reaction)}$$

The metal-catalysed Haber–Weiss reaction may involve the participation of either free iron (or copper) or iron sequestered in the form of nucleotide iron complexes, ferritin, lactoferrin, haemoglobin and myoglobin [16].

Another form of active oxygen, singlet oxygen, can be generated by sensitization of molecules such as riboflavin and its derivatives (FMN, FAD), chlorophyll a and b, bilirubin, retinal, different porphyrins, etc to a specific wavelength of light. Singlet oxygen can also be generated during phagocytosis and by spontaneous dismutation of O_2. [16].

3. Mechanisms for ROS detoxification

To avoid redox imbalance and oxidative DNA damage, a wide array of enzymatic and nonenzymatic antioxidant defences exist. Primary defence mechanisms prevent oxidative damage by scavenging reactive species directly. The primary defence system includes superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and thioredoxin reductase. Secondary defence's combat processes elicited by free radicals. Main compounds belonging to the secondary defence system are ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), β-carotene, vitamin A, NADPH and urate [19, 20, 21, 22 and 23].

Superoxide dismutase (EC 1.15.1.1) destroys the highly reactive radical superoxide by convertion into the less reactive peroxide (H_2O_2), that can be destroyed by catalase or glutathione peroxidase reactions [24 and 25].

$$O_2 \cdot^- + O_2 \cdot^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Catalase (EC 1.11.1.6) is a highly reactive enzyme, reacting with H_2O_2 to form water and molecular oxygen; and with H donors methanol, ethanol, formic acid or phenols [25].

$$\longrightarrow 2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

$$\longrightarrow ROOH + AH_2 \xrightarrow{CAT} H_2O + ROH + A$$

Glutathione peroxidase (EC 1.11.1.19) catalyses the reduction of a variety of hydroperoxides (ROOH and H_2O_2) using GSH, thereby protecting mammalian cells against oxidative damage and, reducing, among others, cellular lipid hydroperoxides [26].

$$\longrightarrow ROOH + 2GSH \xrightarrow{GPX} ROH + GSSG + H_2O$$

The flavin containing thioredoxin reductase (EC 1.6.4.5) is an ubiquitous enzyme able to reduce O_2. and NO by using thioredoxin as a substrate. Transferrine and ferritin sequester iron ions, while ceruloplasmin sequesters copper ions so that the ions are not available to catalyse Haber–Weiss reaction generating .OH or to perform the decomposition of hydroperoxides. Ceruloplasmin has also ferroxidase activity: it oxidizes Fe^{2+} to Fe^{3+} and so inhibits .OH formation from H_2O_2 and iron dependent lipoperoxidation [17].

α-Tocopherol is concentrated inside the membranes, in blood lipoproteins and adrenal glands. It quenches and reacts with $^1O_2$ and is a scavenger of .OH, able to protect membranes from these extremely reactive species. However, its major antioxidant action in biological membranes is to act as a chain breaking antioxidant, donating labile hydrogen to peroxy and alkoxy radicals, thereby breaking the radical chain. It has been proposed that α-TO. may be reduced by ascorbic acid or reduced glutathione. These are scavengers of ROS and other reactive free radicals [27]. β-Carotene is a powerful scavenger of $^1O_2$. Urate binds iron and copper and scavenges .OH, $^1O_2$ and peroxy radicals [16].

4. ROS and activation of apoptosis

In the apoptotic process initial stress-induced damage does not kill cells directly, rather it triggers an apoptotic signalling programme that leads to cell death [28].

Apoptotic cell death is characterized by controlled autodigestion of the cell. This differs from necrosis by distinct morphological and biochemical features, such as chromatin condensation, membrane surface blebbing, oligonucleosomal DNA fragmentation and finally, the breakdown of the cell into a series of smaller units (membrane-bound fragments). These are called apoptotic bodies and in most tissues are phagocytosed by adjacent cells [29]. Such events are associated with activation of specific proteases termed caspases and loss of membrane phospholipid asymmetry resulting in phosphatidylserine externalization [30]. Apoptosis can be initiated by a variety of stimuli, including hyperthermia, growth-factor or hormone withdrawal, glucocorticoids, oxidants, ionizing radiation and multiple classes of chemotherapeutic agents [31 and 32]. Cell viability depends on the type of stress exerted on them. Following an apoptotic signal, cells sustain progressive lipid peroxidation. Thus, ROS and oxidative damage have been implicated in the induction of apoptosis [33, 34, 35 and 36]. The Bcl-2 proto-oncogene is unique among cellular genes for its ability in many contexts to block apoptotic deaths. Moreover, a mechanism has been proposed in which Bcl-2 regulates antioxidant pathways at sites of free radical generation [31]. The protein Bcl-2 protects against apoptosis by blocking cytochrome c release (preventing superoxide production when it is overexpressed) hence this protein may have an antioxidant function [37].

Previously reports suggest that oxygen inhibits the proliferation of human lymphocytes and fibroblasts [38]. Several lines of evidence implicate oxidative stress as a putative mediator of apoptosis. This acts by decreasing intracellular glutathione, the major buffer of the cellular redox status and/or by increasing cellular reactive species [32 and 39]. H_2O_2 at low doses induces apoptosis via production of .OH radicals and alteration of the oxidant/antioxidant pathway [40]. Curiously, similar low doses also cause cell proliferation even in the absence of serum. However, these stimulatory effects do not appear to involve radicals as they are enhanced by inclusion of mannitol or DMSO in the medium [41]. In fact, hydrogen peroxide and superoxide appear to be important regulatory signals. This is suggested by the growth inhibitory effects of CAT and SOD. ROS may contribute a novel redox system of regulatory control superimposed upon established growth signal pathways. Levels of GSH may also be involved in these processes as CAT or SOD treatment of fibroblast increase cellular levels of GSH [42]. In addition, α-tocopherol stimulates growth. Thus, whilst hydrogen peroxide may have a role in promoting the growth of transformed and immortalized cells oxidant protection is important [43]. On the other hand, Murrell (1992) [44] found how free radicals stimulated fibroblast proliferation and Burdon et al. (1996) [45] show that higher oxidant concentrations

not only depress proliferation rates but actually lead to an increase in the appearance of apoptotic-like cells. Inhibitors of GPX, SOD and CAT have a similar effect. Therefore intracellular conditions that are considered more prooxidant than normal, appear to favour apoptosis over proliferation in fibroblasts.

5. Apoptosis, oxidative injury and pathogenesis

O_2 therapy, a widely used component in life-saving intensive care, can cause lung injury, although hyperoxia kills cells via necrosis, not apoptosis [46]. Nevertheless cellular oxidant injury can occur without apoptosis and certain apoptotic mechanisms (i.e. fas-mediated) do not have requirements for ROS [47 and 48], apoptosis, oxidant injury and ROS are strongly related.

Formation of ROS following irradiation is thought to be a major determinant of cellular damage. Recombinant adenoviral vectors expressing the radical-scavenging enzymes Mn-SOD, Cu and Zn-SOD reduce the level of apoptosis [49]. Ferric/ferrous iron via the generation of ROS may mediate the UVB response, finally leading to connective tissue degradation, a hallmark in carcinogenesis, ageing and diseases [50 and 51]. Inorganic iron, in concert with chemical and physical inducers of the heat shock response, may trigger apoptosis. Accumulation of iron in injured tissue may thereby predispose to accelerated apoptosis and account in part for poor wound healing and organ failure [52].

Alkalosis is a clinical complication resulting from various pathological and physiological conditions. Although it is well established that reducing the cellular proton concentration is lethal, the mechanism leading to cell death is unknown. Mitochondrial respiration generates a proton gradient and superoxide radicals, suggesting a possible link between oxidative stress, mitochondrial integrity and alkaline-induced cell death [53]. Manganese superoxide dismutase removes superoxide radicals in mitochondria and thus protects mitochondria from oxidative injury. Therefore, over expression of manganese superoxide dismutase reduced the levels of intracellular reactive oxygen species and prevents cell death [54].

Myeloid cells are a major source of superoxide and other oxygen metabolites. As a protective mechanism, cells express antioxidant enzymes such as Mn-SOD, Cu, Zn-SOD and GPX. Myeloid leukaemic lines, normal peripheral blood lymphocytes and monocytes are sensitive to cytotoxic effects of tumour necrosis factor (TNF) dramatically increased their levels of Mn-SOD RNA in the presence of TNF. In contrast Cu, Zn-SOD and GPX RNA levels do not

increase in these cells. Kizaki et al., (1993) [55] reported that Mn-SOD may provide protection against cytotoxicity of TNF in hematopoietic cells. TNF-induced antiproliferative effects and caspase-3 activation, indicators of apoptosis are also completely suppressed by transfection of cells with Mn-SOD. Suppression of apoptosis induced by okadaic acid, hydrogen peroxide and taxol is inhibited by Mn-SOD but not that induced by vincristine, vinblastine or daunomycin. Activation of the p53-mediated DNA damage response induces either G1 cell cycle arrest or apoptosis. Data suggest that p21 may serve as a critical checkpoint regulator during the p53-mediated DNA damage response [56]. Overall, these results demonstrate that in addition to several recently identified signalling molecules, reactive oxygen intermediates play a critical role in activation of $NF_{\kappa}B$, activated protein-1, c-Jun kinase and apoptosis induced by TNF and other agents (Manna et al., 1998).

Gotoh and Cooper (1998) [57] suggest that TNF-induced activation of the apoptosis signal-regulating kinase 1 (ASK1) is mediated by ROS. They examine how ASK1 activity is regulated by ROS and find that ASK1 forms dimers or higher order oligomers in 293 cells. TNF or hydrogen peroxide treatment increases the dimeric form of ASK1, whilst pretreatment with N-acetylcysteine reduces it. However, synthetic dimerization of an ASK1-gyrase B fusion protein by coumermycin results in substantial activation of ASK1. This suggest that dimerization of ASK1 is sufficient for its activation. We may deduce from these results that TNF causes ASK1 activation via ROS-mediated dimerization.

The antioxidant superoxide dismutase but not catalase inhibited apoptosis induced by either oxidised low-density lipoproteins (oxLDL) or 25-hydroxycholesterol. This suggests not only that superoxide plays an important role but that a critical interaction between oxLDL and the cell takes place on the outer surface of the membrane, because superoxide dismutase is not membrane-permeable [58].

TGFβ may play an important part in the inhibition of cell proliferation and in the regulation of apoptosis. This may be induced by TGFβ preceded by reduction in p26-Bcl-2 protein levels. Therefore, TGFβ regulates Bcl-2 expression in adenoma cells undergoing apoptosis in response to TGFβ [59].

Apoptosis of neutrophils may be mediated by endogenous oxidative products. This suggestion is confirmed by observation that apoptosis of normal neutrophils is markedly inhibited by reduction of intracellular hydrogen peroxide levels. Inhibition of apoptosis in normal neutrophils by addition of catalase also occurs [60]. Activation of cell death is blocked by a variety of antioxidants. Although reactive oxygen intermediates do not act as

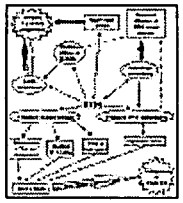
mediators in the execution phase of CD95-mediated apoptosis, they are involved in the transcriptional regulation of CD95L expression [61]. A potential role of CD in oxidative stress-mediated cell death, ischemia/reperfusion and other diseases characterised by a disturbed redox balance has been recently reported [62]. Caspar-Bauguil et al. (1998) [63] show that activated T-lymphocytes are present in early atherosclerotic lesions where they may interact with oxLDLs. They concluded that mildly oxidized LDLs inhibit the proliferation and CD25 expression of activated T-lymphocytes. This suggests that oxLDLs may slow down the T-cell response in atherosclerotic lesions.

Programmed cell death produced by the spermidine/spermine *N*1-acetyltransferase-inducing polyamine analogues can be delayed by the inhibition of polyamine catabolism or the expression of the Bcl-2. Natural polyamines may stimulate malignant transformation of immortalised cells [64]. These organic polycations have two opposing functions. First, the production of ROS during the catabolism of polyamines leading to programmed cell death. This shows how a decrease of intracellular spermine levels is involved in sensitization towards apoptosis induced by TNF [65]. Secondly, spermine has the ability to act directly as a free radical scavenger protecting DNA from ROS species attack [66 and 67].

6. Antioxidants against tumours

Antioxidant enzymes can antagonize initiation and promotion phases of carcinogenesis and they are reduced in many malignancies. The most commonly decreased enzyme is the mitochondrial Mn-SOD. This has led to suggestions that Mn-SOD might be a new type of tumour-suppressor gene. However, observations tend to ascribe the deficiency of the Mn-SOD activity to a defect in the expression of the gene rather than to its deletion. Transition metals (Mn, Fe) have been found to be highly deficient in some tumours. It is proposed that in the early stage of carcinogenesis an impairment of the signal transduction machinery might cause the defect in the Mn-SOD gene expression. Owing to a second messenger function of ROS activating transcription factors. Combined with the ability of Mn to facilitate the dismutation of $O_2^{\cdot}$ to H_2O_2 and Fe's participation in the Fenton reaction. This may result in the limitation to binding of transcription factors like AP-1 and $NF_{\kappa}B$ to the DNA as a consequence of the metal deficiency [68].

The generation of large amounts of reactive oxygen intermediates (as shown in Fig. 2) may contribute to the ability of some tumours to mutate, inhibit antiproteases and injure local tissues. Therefore promoting tumour heterogeneity, invasion and metastasis [1 and 69].



(21K)

Fig. 2. Under normal conditions, the cell is able to detoxify radicals but when ROS accumulate a number of changes occur in macromolecules and cellular environment, which can lead to the accumulation of oncogenic mutations and thus contribute to the pathogenesis of cancer and tumour promotion. As shown in the figure, chemical modification of DNA cause a change in their hydrogen bonding specificity, ring-opened purines and pyrimidines fragmentation products block DNA replication and conformational changes in DNA diminish the accuracy of replication by DNA polymerases. On the other hand, oxidative damage to proteases and oxidative injury to local tissues may promote tumour progression and metastasis.

Several recent results suggest temporal relationships between oxidative stress, genomic instability and the development of cancer [70, 71, 72 and 73]. Free radicals may induce several DNA sequence changes: point mutations, deletions, gene amplification and rearrangements that result in the activation of several proto-oncogenes and/or the activation of some tumour suppressor genes [74].

In support of this, the steady state levels of one or more base damage products have been observed in DNA isolated from cancerous tumour biopsies of human lung, colon, kidney, breast, liver and bladder. DNA repair is also responsible for one of the most common cancers, hereditary nonpolyposis colon cancer [1].

DNA damage by ROS can cause multiple lesions, including single and double strand breaks and modified pyrimidines and purines. Repair of these lesions occurs primarily by means of base excision although nucleotide excision repair may also be involved. There are several different pathways leading from initial DNA base damage by ROS to subsequent mutation [1].

The simplest one is the chemical modification of base DNA. Additionally, ring-opened purines and a number of pyrimidine fragmentation products can block replication and may thus be mutagenic (Fig. 2). Singlet oxygen induced DNA damage is targeted selectively at

guanine residues. DNA polymerase is known to be sensitive to damage-induced errors at guanines. The contribution of oxidative damage to polymerase-specific 'hot-spots', which is probably the major contributor to DNA polymerase mediated mutagenesis, is possibly a second mechanism. A third mechanism is linked to conformational change in the DNA template (Fig. 2). Although direct studies of the effect of base modifications on DNA conformation are just commencing, it is known that many oxidized bases are nonplanar and could change local DNA structure [1].

Observations of various types of cancer, present a possible link between decreased activities of antioxidant enzymes and increased levels of hydroxylated DNA base, due to oxidative damage. Supporting the idea that active oxygen may be increased in tumoural cells (Table 1). In fact, the levels of the antioxidant enzymes glutathione peroxidase, catalase and superoxide dismutase in lymphocytes of acute lymphoblastic leukaemia (ALL) patients are lower than in lymphocytes of controls [77]. In addition, the individual kinetic of DNA repair varies significantly between specimens derived from healthy individuals and chronic lymphatic leukaemia (CLL) patients; large differences are also found in the DNA repair half-time ($t_{1/2}$). Methoxyamine is a DNA repair modifier, which blocks the base excision repair pathway. Pretreatment of cells with this agent reveals a similar base excision repair/independent DNA incision in almost all normal lymphocyte samples. In contrast, this portion varies to a great extent both relatively and absolutely among individual samples of CLL lymphocytes, suggesting a loss of stringent control of DNA repair processes in these cells.

Table 1. Tumours which have been strongly related with the oxidant-antioxidant imbalance

Cancer	Key references
Bladder	[1]
Blood	[75–77]
Bowel	[1,74]
Breast	[1,78]
Colorectum	[1,79,80]
Liver	[1]
Lung	[1,72]
Kidney	[1,81]
Oesophagus	[82]
Ovary	[29]
Prostate	[83]
Skin	[84]

The fact that a reduced activity of the selenium-dependent enzyme glutathione peroxidase in blood is associated with an increased risk and poor prognosis of cancer in humans is still controversial [85]. An association between low selenium level and advanced tumour disease exists, but it yet to be decided whether this phenomenon is more likely to be a consequence or a causative factor for development and course of the disease [86]. In neoplastic human cell lines, two bipolar factors appear to influence the activities of CAT, Mn-SOD, GPX, Cu and Zn-SOD. Potentially low superoxide production and intrinsically low peroxidizability of tumour cell membranes underlie the peculiar variation of antioxidant enzyme activities in tumour cells [87].

Macrophages have two mechanisms for destroying cancer cells: one mediated by proteolytic activity and a second that depends on the generation of oxygen-derived free radicals [88]. Digitonin stimulates activated macrophages to produce superoxide, hydrogen peroxide and possibly other free radicals that can increase macrophage-induced tumour cell cytotoxicity [89].

Results hint that H_2O_2 may act as a physiological mediator of intracellular response or as a second messenger in mammalian cells. In fact, H_2O_2 has been implicated as an indirect activator of $NF_{\kappa}B$ [8 and90]. Over expression of manganese superoxide dismutase in human

breast cancer MCF-7 cells completely abolishes TNF-mediated $NF_{\kappa}B$ activation, $I_{\kappa}B$ degradation, p65 nuclear translocation and $NF_{\kappa}B$-dependent reporter gene expression. Besides TNF, phorbol ester-, okadaic acid-, ceramide- and LPS-induced activation of $NF_{\kappa}B$ is blocked by Mn-SOD, indicating a common pathway of activation. Inhibition of both $NF_{\kappa}B$ binding activity and oxidative DNA damage hint that its antioxidant potential outweighs its oxidative capacity in a cellular environment. This may contribute to anticarcinogenic effects [10]. Additionally, Mn-SOD blocks the TNF mediated activation of activated protein-1, stress-activated c-Jun protein kinase and mitogen-activated protein kinase [91].

Expression of the genes encoding antioxidant enzymes Mn-SOD and GPX are increased in the lungs of influenza virus infected animals, whereas Cu, Zn-SOD and CAT mRNA are not induced by viral infection. Activation of the transcriptional regulatory proteins AP-1, C/EBP and $NF_{\kappa}B$ (which are known to be affected by oxidant stress) is demonstrated by electrophoretic mobility shift assay after viral infection [92].

Described above, oxidants can trigger the activation of multiple signalling pathways that influence the cytotoxicity observed in affected cells. This includes phosphorylation cascades, leading to the activation of MAPKs, $NF_{\kappa}B$ [93] and a multiprotein complex that regulates a variety of genes important for immunity, inflammation and cancer. Activation of these genes, induced by silica, lipopolysaccharide (LPS) and PMA, is blocked by catalase [94].

Oxidative processes in tumour cells may have a strong influence on the host response against tumours. Thus, in H-2kb-transformed tumour cells reduction of superoxide is associated with a significant increase in the level of Cu, Zn-SOD and GPX and a reduction in the DNA-binding form of $NF_{\kappa}B$ [95].

Mn-SOD is reduced in a variety of tumour cells and has been proposed to be a new type of tumour suppressor gene. The mechanism(s) by which Mn-SOD suppresses cancer development is currently unknown. However, expression of this antioxidant might play a significant role in maintaining cellular redox status. The relationship between Mn-SOD expression, modulation of DNA-binding activity, transcriptional activation of redox-sensitive oncoproteins and tumour suppressor proteins has been recently studied [96]. Electrophoretic mobility shift assay and transcriptional activation studies revealed an inverse correlation between Mn-SOD expression and activity of c-Jun-associated transcription factors, activator protein 1 and c-AMP-responsive element binding protein. Expression of Bcl-x_L (an activator protein 1 target gene and antiapoptotic member of the Bcl-2 family) is decreased in Mn-SOD-transfected cell lines. Thus, over expression of Mn-SOD may exert its tumour

suppressor activity in part by modulation of specific oncogenes [97].

7. Directions for future research

Critical steps in the signal transduction cascade are sensitive to oxidants and antioxidants. At least two well-defined transcription factors ($NF_{\kappa}B$ and AP-1) have been identified to be regulated by the intracellular redox state. Binding sites of these redox-regulated transcription factors are located in the promoter region of a large variety of genes that are directly involved in the pathogenesis of cancer and other diseases. Biochemical and clinical studies indicate that antioxidant therapy may be useful in the treatment of several diseases [98]. Moreover, a number of structurally different anticarcinogenic agents inhibit ROS production and oxidative DNA damage as they inhibit inflammation and tumour promotion [99]. The above statements underline the importance of ROS and oxidative genetic damage to the carcinogenic process. Also pointing to the possibility that some types of cancer may be preventable if the cycles of tumour promotion can be interrupted [100]. The mechanism through which ROS plays an important role in the initiation and progression of cancer and its ability to induce apoptosis is not yet fully understood. Therefore, further efforts are also necessary to fully elucidate the importance of free radical scavengers in the therapy of several diseases. Therefore we consider it important to continue investigation on the biochemical roles of these antioxidant enzymes which clearly related to among other cellular processes both apoptosis and pathogenesis of cancer.

Acknowledgements

We wish to thank I. Núñez de Castro and M.A. Medina for critically reading the manuscript, N. McVeigh and C. Segura for the revision of the spelling and the grammar and post-graduate students C. Pérez-Gómez, R. Rosado and J.M. Segura for their help in the bibliographic search. This work was supported by project SAF98-0150 (Ministry of Education, Spain). To the memory of Emilio Matés Sánchez.

References

1. H. Wiseman and B. Halliwell, Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem. J.* **313** (1996), pp. 17–29.

Abstract-MEDLINE | Abstract-Elsevier BIOBASE | Abstract-EMBASE

2. C.R. Hunt, J.E. Sim, S.J. Sullivan, T. Featherstone, W. Golden, C.V. Kapp-Herr, R.A. Hock, R.A. Gomez, A.J. Parsian and D.R. Spitz, Genomic instability and catalase gene amplification induced by chronic exposure to oxidative stress. *Cancer Res.* **58** (1998), pp. 3986–3992. Abstract-MEDLINE | Abstract-EMBASE

3. E.M. Mills, K. Takeda, Z.X. Yu, V. Ferrans, Y. Katagiri, H. Jiang, M.C. Lavigne, T.L. Leto and G. Guroff, Nerve growth factor treatment prevents the increase in superoxide produced by epidermal growth factor in PC12 cells. *J. Biol. Chem.* **273** (1998), pp. 22165–22168. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

4. E. Rollet-Labelle, M.J. Grange, C. Elbim, C. Marquetty, M.A. Gougerot-Pocidalo and C. Pasquier, Hydroxyl radicals as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. *Free Rad. Biol. Med.* **24** (1998), pp. 563–572. SummaryPlus | **Full Text + Links** | PDF (927 K)

5. K. Tanaka, J.B. Pracyk, K. Takeda, Z.X. Yu, V.J. Ferrans, S.S. Deshpande, M. Ozaki, P.M. Hwang, C.J. Lowenstein, K. Irani and T. Finkel, Expression of Id1 results in apoptosis of cardiac myocytes through a redox-dependent mechanism. *J. Biol. Chem.* **273** (1998), pp. 25922–25928. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

6. R.A. Floyd, The role of 8-hydroxydeoxyguanosine in carcinogenesis. *Carcinogenesis* **11** (1990), pp. 1447–1450. Abstract-MEDLINE | Abstract-EMBASE

7. K.Z. Guyton, Y. Liu, M. Gorospe, Q. Xu and N.J. Holbrook, Activation of mitogen-activated protein Kinase by H_2O_2: role in cell survival following oxidant injury. *J. Biol. Chem.* **271** (1996), pp. 4138–4142. Abstract-MEDLINE | Abstract-Elsevier BIOBASE | Abstract-EMBASE

8. Y.S. Bae, S.W. Kang, M.S. Seo, I.C. Baines, E. Tekle, P.B. Chock and S.G. Rhee, Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. *J. Biol. Chem.* **272** (1997), pp. 217–221. Abstract-MEDLINE

9. S.K. Manna, H.J. Zhang, T. Yan, L.W. Oberley and B.B. Aggarwal, Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-b and activated protein-1. *J. Biol. Chem.* **273**

(1998), pp. 13245–13254. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

10. C.A. Musonda and J.K. Chipman. *Quercetin inhibits hydrogen peroxide (H_2O_2)-induced $NF_{\kappa}B$ DNA binding activity and DNA damage in HepG2 cells, Carcinogenesis* **19** (1998), pp. 1583–1589. Abstract-MEDLINE | **Full Text** via CrossRef

11. C.L. Limoli, A. Hartmann, L. Shephard, C. Yang, D.A. Boothman, J. Bartholomew and W.F. Morgan, Apoptosis, reproductive failure and oxidative stress in chinese hamster ovary cells with compromised genomic integrity. *Cancer Res.* **58** (1998), pp. 3712–3718. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

12. M. Behn, S. Qun, W. Pankow, K. Havemann and M. Schuermann, Frequent detection of ras and p53 mutations in brush cytology samples from lung cancer patients by a restriction fragment length polymorphism-based 'enriched PCR' technique. *Clin. Cancer Res.* **4** (1998), pp. 361–371. Abstract-MEDLINE | Abstract-EMBASE

13. F. Eisinger, J. Jacquemier, C. Charpin, D. Stoppa-Lyonnet, B. Bressac-de-Paillerets, J.P. Peyrat, M. Longy, J.M. Guinebretiere, R. Sauvan, T. Noguchi, D. Birnbaum and H. Sobol, Mutations at BRCA1: the medullary breast carcinoma revisited. *Cancer Res.* **58** (1998), pp. 1588–1592. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

14. S. Gretarsdottir, S. Thorlacius, R. Valgardsdottir, S. Gudlaugsdottir, S. Sigurdsson, M. Stenarsdottir, J.G. Janasson, K. Anamthawat-Johsson and J.E. Eyfjörd, BRCA2 and p53 mutations in primary breast cancer in relation to genetic instability. *Cancer Res.* **58** (1998), pp. 859–862. Abstract-MEDLINE | Abstract-EMBASE

15. A.R. Cross and O.T.G. Jones, Enzymic mechanisms of superoxide production. *Biochem. Biophys. Acta* **1057** (1991), pp. 281–298. Abstract-MEDLINE | Abstract-EMBASE

16. M. Picardo and S. Passi, Free radicals. In: J.D. Bos Editor, *Skin immune system (SIS)* CRC Press, Boca Raton, New York (1997), pp. 207–226.

17. C.W. Trenam, D.R. Blake and C. Morris, Skin inflammation: reactive oxygen species and the role of iron. *J. Invest. Dermatol.* **99** (1992), pp. 675–682. Abstract-MEDLINE | Abstract-EMBASE

18. J.M. Gutteridge, G.J. Quinlan and P. Kovacic, Phagomimetic action of antimicrobial agents. *Free Radic. Res.* **1** (1998), pp. 1–14. Abstract-MEDLINE | Abstract-EMBASE

19. J.L. Beaudeux, M. Gardes-Albert, J. Delattre, A. Legrand, F. Rousselet and J. Peynet, Resistance of lipoprotein(a) to lipid peroxidation induced by oxygenated free radicals produced by gamma radiolysis: a comparison with low-density lipoprotein. *Biochem. J.* **314** (1996), pp. 277–284. Abstract-EMBASE | Abstract-MEDLINE | Abstract-Elsevier BIOBASE

20. S.E. Stait and D.S. Leake, The effects of ascorbate and dehydroascorbate on the oxidation of low-density lipoprotein. *Biochem. J.* **320** (1996), pp. 373–381. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

21. L. Hall, K. Williams, A.C.F. Perry, J. Frayne and J.A. Jury, The majority of human glutathione peroxidase type 5 (GPX5) transcripts are incorrectly spliced: implications for the role of GPX5 in the male reproductive tract. *Biochem. J.* **333** (1998), pp. 5–9. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

22. R.A. Patterson and D.S. Leake, Human serum, cysteine and histidine inhibit the oxidation of low density lipoprotein less at acidic pH. *FEBS Lett.* **434** (1998), pp. 317–321. SummaryPlus | **Full Text + Links** | PDF (170 K)

23. W. Stahl, A. Junghans, B. de Boer, E.S. Driomina, K. Brivida and H. Sies, Carotenoid mixtures protect multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. *FEBS Lett.* **427** (1998), pp. 305–308. SummaryPlus | **Full Text + Links** | PDF (64 K)

24. I. Fridovich, Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem.* **64** (1995), pp. 97–112. Abstract-MEDLINE | Abstract-Elsevier BIOBASE | Abstract-EMBASE

25. J.M. Matés and F. Sánchez-Jiménez, Antioxidant enzymes and their implications in pathophysiologic processes. *Front. Biosc.* **4** (1999), pp. 339–345.

26. L. Jornot, H. Petersen and A.F. Junod, Hydrogen peroxide-induced DNA damage is independent of nuclear calcium but dependent on redox-active ions. *Biochem. J.* **335** (1998), pp. 85–94. Abstract-EMBASE | Abstract-MEDLINE

27. J.E. Packer, T.F. Slater and R.L. Wilson, Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* **278** (1979), pp. 737–739.

28. V.L. Gabai, A.B. Meriin, J.A. Yaglom, V.Z. Volloch and M.Y. Sherman, Role of Hsp70 in regulation of stress-kinase JNK: implications in apoptosis and aging. *FEBS Lett.* **438** (1998), pp. 1–4. SummaryPlus | **Full Text + Links** | PDF (65 K)

29. C.B. Thompson, Apoptosis in the pathogenesis and treatment of disease. *Science* **267** (1995), pp. 1456–1462. Abstract-MEDLINE | Abstract-Elsevier BIOBASE | Abstract-EMBASE

30. J.P. Fabisiak, Y.Y. Tyurina, A.A. Tyurin, J.S. Lazo and V.E. Kagan, Ramdom versus selective membrane phospholipid oxidation in apoptosis: role of phosphatidylserine. *Biochemistry* **37** (1998), pp. 13781–13790. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

31. D.M. Hockenbery, Z.N. Oltvai, X.M. Yin, C.L. Milliman and S.J. Korsmeyer, Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* **75** (1993), pp. 241–251. Abstract-MEDLINE | Abstract-EMBASE

32. H.K. Bojes, K. Datta, J. Xu, A. Chin, P. Simonian, G. Nuñez and J.P. Kehrer, Bcl-xL overexpression attenuates glutathione depletion in FL5.12 cells following interleukin-3 withdrawal. *Biochem. J.* **325** (1997), pp. 115–119.

33. P. Amstad, R. Moret and P. Cerutti, Glutathione peroxidase compensates for the hypersensitivity of Cu,Zn-superoxide dismutase overproducers to oxidant stress. *J. Biol. Chem.* **269** (1994), pp. 1606–1609. Abstract-MEDLINE | Abstract-Elsevier BIOBASE | Abstract-EMBASE

34. S. Czene, M. Tibäck and M. Harms-Ringdahl, pH-dependent DNA cleavage in permeabilized human fibroblasts. *Biochem. J.* **323** (1997), pp. 337–341. Abstract-MEDLINE | Abstract-EMBASE

35. S. Dimmeler, J. Haendeler, A. Sause and A.M. Zeiher, Nitric oxide inhibits APO-1/Fas-mediated cell death. *Cell Growth Differ.* **9** (1998), pp. 415–422. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

36. J. Tamarit, E. Cabiscol and J. Ros, Identification of the major oxidatively damaged proteins in *Escherichia coli* cells exposed to oxidative stress. *J. Biol. Chem.* **273** (1998), pp. 3027–3032. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

37. J. Cai and D.P. Jones, Superoxide in apoptosis: Mitochondrial generation triggered by cytochrome c loss. *J. Biol. Chem.* **273** (1998), pp. 11401–11404. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

38. I. Karlberg, K. Lindahl-Kiessling, H. Löw and A. Mattsson, Role of aerobic conditions in the control of cell proliferation. *Int. Arch. Allergy Appl. Immunol.* **65** (1981), pp. 250–256. Abstract-EMBASE | Abstract-MEDLINE

39. Y. Suzuki, Y. Ono and Y. Hirabayashi, Rapid and specific reactive oxygen species generation via NADPH oxidase activation during Fas-mediated apoptosis. *FEBS Lett.* **425** (1998), pp. 209–212. SummaryPlus | **Full Text + Links** | PDF (120 K)

40. B.J. Toledano, Y. Bastien, F. Noya, S. Baruchel and B. Mazer, Platelet-activating factor abrogates apoptosis induced by cross-linking of the surface IgM receptor in a human B lymphoblastoid cell line. *J. Immunol.* **158** (1997), pp. 3705–3715. Abstract-MEDLINE

41. R.H. Burdon, V. Gill and C. Rice-Evans, Cell proliferation and oxidative stress. *Free Radic. Res. Commun.* **7** (1989), pp. 149–159. Abstract-MEDLINE

42. R.H. Burdon, D. Alliangana and V. Gill, Endogenously generated active oxygen species and cellular glutathione levels in relation to BHK-21 cell proliferation. *Free Radic.Res.* **211** (1994), pp. 21–133.

43. R.H. Burdon, V. Gill and C. Rice-Evans, Oxidative stress and tumour cell proliferation. *Free Radic. Res. Commun.* **11** (1990), pp. 65–76. Abstract-EMBASE | Abstract-MEDLINE

44. G.A. Murrell, An insight into Dupuytren's contracture. *Ann. Roy. Coll. Surg. Engl.* **74** (1992), pp. 156–160.

45. R.H. Burdon, V. Gill and D. Alliangana, Hydrogen peroxide in relation to proliferation and apoptosis in BHK-21 hamster fibroblasts. *Free Radic. Res.* **24** (1996), pp. 81–93. Abstract-MEDLINE | Abstract-Elsevier BIOBASE

46. J.A. Kazzaz, J. Xu, T.A. Palaia, L. Mantell, A.M. Fein and S. Horowitz, Cellular oxygen toxicity. Oxidant injury without apoptosis. *J. Biol. Chem.* **271** (1996), pp. 15182–15186. Abstract-MEDLINE | Abstract-EMBASE

47. A. Vassilev, Z. Ozer, C. Navara, S. Mahajan and F.M. Uckun, Bruton's tyrosine kinase as an inhibitor of the Fas/CD95 death-inducing signaling complex. *J. Biol. Chem.* **274** (1999), pp. 1646–1656. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

48. M.I. Gutierrez, B. Cherney, A. Hussain, H. Mostowski, G. Tosato, I. Magrath and K. Bhatia, Bax is frequently compromised in Burkitt's lymphomas with irreversible resistance to Fas-induced apoptosis. *Cancer Res.* **59** (1999), pp. 696–703. Abstract-EMBASE | Abstract-MEDLINE

49. R.M. Zwacka, L. Dudu, M.W. Epperly, J.S. Greenberger and J.F. Engelhardt, Redox gene therapy protects human IB-3 lung epithelial cells against ionizing radiation-induced apoptosis. *Hum. Gene Ther.* **9** (1998), pp. 1381–1386. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

50. P. Brenneisen, J. Wenk, L.O. Klotz, M. Wlaschek, K. Briviba, T. Krieg, H. Sies and K. Scharffetter-Kochanek, Central role of Ferrous/Ferric iron in the ultraviolet B irradiation-mediated signaling pathway leading to increased interstitial collagenase (matrix-degrading metalloprotease (MMP)-1) and stromelysin-1 (MMP-3) mRNA levels in cultured human dermal fibroblasts. *J. Biol. Chem.* **273** (1998), pp. 5279–5287. Abstract-EMBASE | Abstract-MEDLINE | **Full Text** via CrossRef

51. A. Valavanidis, H. Balomenou, I. Macropoulou and I. Zarodimos, A study of the synergistic interaction of asbestos fibers with cigarette tar extracts for the generation of hydroxyl radicals in aqueous buffer solution. *Free Radic. Biol. Med.* **20** (1996), pp. 853–858. SummaryPlus | **Full Text + Links** | PDF (549 K)

52. A.K. Jacob, R.S. Hotchkiss, S.L. DeMeester, M. Hiramatsu, I.E. Karl, P.E. Swanson, J.P. Cobb and T.G. Buchman, Endothelial cell apoptosis is accelerated by inorganic iron and heat via an oxygen radical dependent mechanism. *Surgery* **122** (1997), pp. 243–254. Abstract-EMBASE | Abstract-MEDLINE

53. J. Duranteau, N.S. Chandel, A. Kulisz, Z. Shao and P.T. Schumacker, Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. *J. Biol. Chem.* **273**

(1998), pp. 11619–11624. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

54. H.J. Majima, T.D. Oberley, K. Furukawa, M.P. Mattson, H.C. Yen, L.I. Szweda and D.K.S. Clair, Prevention of mitochondrial injury by manganese superoxide dismutase reveals a primary mechanism for alkaline-induced cell death. *J. Biol. Chem.* **273** (1998), pp. 8217–8224. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

55. M. Kizaki, A. Sakashita, A. Karmakar, C.W. Lin and H.P. Koeffler, Regulation of manganese superoxide dismutase and other antioxidant genes in normal and leukaemic hematopoietic cells and their relationship to cytotoxicity by tumor necrosis factor. *Blood* **82** (1993), pp. 1142–1150. Abstract-MEDLINE | Abstract-EMBASE

56. J.L.M. Gervais, P. Seth and H. Zhang, Cleavage of CDK inhibitor p21Cip1/Waf1 by caspases is an early event during DNA damage-induced apoptosis. *J. Biol. Chem.* **273** pp. 7–19212.

57. Y. Gotoh and J.A. Cooper, Reactive oxygen species and dimerization-induced activation of apoptosis signal-regulating kinase 1 in tumor necrosis factor-alpha signal transduction. *J. Biol. Chem.* **273** (1998), pp. 17477–17482. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

58. M. Harada-Shiba, M. Kinoshita, H. Kamido and K. Shimokado, Oxidized low density lipoprotein induces apoptosis in cultured human umbilical vein endothelial cells by common and unique mechanisms. *J. Biol. Chem.* **273** (1998), pp. 9681–9687. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

59. A. Hague, T.S. Bracey, D.J. Hicks, J.C. Reed and C. Paraskeva, Decreased levels of p26-Bcl-2, but not p30 phosphorylated Bcl-2, precede TGFβ1-induced apoptosis in colorectal adenoma cells. *Carcinogenesis* **19** (1998), pp. 1691–1695. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

60. Y. Kasahara, K. Iwai, A. Yachie, K. Ohta, A. Konno, H. Seki, T. Miyawaki and N. Taniguchi, Involvement of reactive oxygen intermediates in spontaneous and CD95 (Fas/APO-1)-mediated apoptosis of neutrophils. *Blood* **89** (1997), pp. 1748–1753. Abstract-MEDLINE

61. M.K.A. Bauer, M. Vogt, M. Los, J. Siegel, S. Wesselborg and K. Schulze-Osthoff, Role of reactive oxygen intermediates in activation-induced CD95 (APO-1/Fas) ligand expression. *J. Biol. Chem.* **273** (1998), pp. 8048–8055. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

62. M. Vogt, M.K.A. Bauer, D. Ferrari and K. Schulze-Osthoff, Oxidative stress and hypoxia/reoxygenation trigger CD95 (APO-1/Fas) ligand expression in microglial cells. *FEBS Lett.* **429** (1998), pp. 67–72. SummaryPlus | **Full Text + Links** | PDF (245 K)

63. S. Caspar-Bauguil, M. Saadawi, A. Negre-Salvayre, M. Thomsen, R. Salvayre and H. Benoist, Mildly oxidized low-density lipoproteins suppress the proliferation of activated CD4+ T-lymphocytes and their interleukin 2 receptor expression in vitro. *Biochem. J.* **330** (1998), pp. 659–666. Abstract-MEDLINE | Abstract-EMBASE

64. A. Tabib and U. Bachrach, Polyamines induce malignant transformation in cultured NIH 3T3 fibroblasts. *Int. J. Biochem. Cell Biol.* **30** (1998), pp. 135–146. SummaryPlus | **Full Text + Links** | PDF (955 K)

65. L.C. Penning, R.G. Schipper, D. Vercammen, A.A. Verhofstad, T. Denecker, R. Beyaert and P. Vandenabeele, Sensitization of tnf-induced apoptosis with polyamine synthesis inhibitors in different human and murine tumour cell lines. *Cytokine* **10** (1998), pp. 423–431. Abstract | PDF (206 K)

66. H.C. Ha, N.S. Sirisoma, P. Kuppusamy, J.L. Zweier, P.M. Woster and R.A. Casero, Jr, The natural polyamine spermine functions directly as a free radical scavenger. *Proc. Natl. Acad. Sci.* **95** (1998), pp. 11140–11145. Abstract-MEDLINE | **Full Text** via CrossRef

67. H.C. Ha, P.M. Woster and R.A. Casero, Jr, Structural specificity of polyamines and polyamine analogues in the protection of DNA from strand breaks induced by reactive oxygen species. *Biochem. Biophys. Res. Commun.* **244** (1998), pp. 298–303. Abstract | **Abstract + References** | PDF (296 K)

68. S. Borrello, M.E. De Leo and T. Galeotti, Defective gene expression of MnSOD in cancer cells. *Mol. Aspects Med.* **14** (1993), pp. 253–258. Abstract

69. T.P. Szatrowski and C.F. Nathan, Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Res.* **51** (1991), pp. 794–798. Abstract-MEDLINE | Abstract-

EMBASE

70. P.C. Dedon, J.P. Plastaras, C.A. Rouzer and L.J. Marnett, Indirect mutagenesis by oxidative damage: formation of the pyrimidopurinone adduct of deoxyguanosine by base propenal. *Proc. Natl. Acad. Sci.* **95** (1998), pp. 11113–11116. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

71. Y. Hiraku, M. Yamasaki and S. Kawanishi, Oxidative DNA damage induced by homogentisic acid, a tyrosine metabolite. *FEBS Lett.* **432** (1998), pp. 13–16. SummaryPlus | **Full Text + Links** | PDF (151 K)

72. C.H. Kennedy, R. Cueto, S.A. Belinsky, J.F. Lechner and W.A. Pryor, Overexpression of hMTH1 mRNA: a molecular marker of oxidative stress in lung cancer cells. *FEBS Lett.* **429** (1998), pp. 17–20. SummaryPlus | **Full Text + Links** | PDF (114 K)

73. R.A. Vora, A.E. Pegg and S.E. Ealick, A new model for how O6-methylguanine-DNA methyltransferase binds DNA. *Proteins* **32** (1998), pp. 3–6. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

74. T. Tanaka, K. Kawabata, M. Kakumoto, A. Hara, A. Murakami, W. Kuki, Y. Takahashi, H. Yonei, M. Maeda, T. Ota, S. Odashima, T. Yamane, K. Koshimizu and H. Ohigashi, Citrus auraptene exerts dose-dependent chemopreventive activity in rat large bowel tumorigenesis: the inhibition correlates with suppression of cell proliferation and lipid peroxidation and with induction of phase II drug-metabolizing enzymes. *Cancer Res.* **58** (1998), pp. 2550–2556. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

75. C. Buschfort, M.R. Müller, S. Seeber, M.F. Rajewsky and J. Thomale, DNA excision repair profiles of normal and leukemic human lymphocytes: functional analysis at the single-cell level. *Cancer Res.* **57** (1997), pp. 651–658. Abstract-EMBASE | Abstract-MEDLINE

76. M.R. Müller, C. Buschfort, J. Thomale, C. Lensing, M.F. Rajewsky and S. Seeber, DNA repair and cellular resistence to alkylating agents in chronic lymphocytic leukemia. *Clin. Cancer Res.* **3** (1997), pp. 2055–2061. Abstract-EMBASE | Abstract-MEDLINE

77. S. Sentüker, B. Karahalil, M. Inal, H. Yilmaz, H. Müslümanoglü, G. Gedikoglu and M. Dizdaroglu, Oxidative DNA base damage and antioxidant enzyme levels in childhood acute

lymphoblastic leukemia. *FEBS Lett.* **416** (1997), pp. 286–290.

78. Y.J. Lee, S.S. Galoforo, C.M. Berns, J.C. Chen, B.H. Davis, J.E. Sim, P.M. Corry and D.R. Spitz, Glucose deprivation-induced cytotoxicity and alterations in mitogen-activated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells. *J. Biol. Chem.* **273** (1998), pp. 5294–5299. Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

79. R. Chinery, R.D. Beauchamp, Y. Shyr, S.C. Kirkland and R.J.D. Coffey, Antioxidants reduce cyclooxygenase-2 expression, prostaglandin production and proliferation in colorectal cancer cells. *Cancer Res.* **58** (1998), pp. 2323–2327. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

80. H. Yamamoto, H. Sawai, T.K. Weber, M.A. Rodriguez-Bigas and M. Perucho, Somatic frameshift mutations in DNA mismatch repair and proapoptosis genes in hereditary nonpolyposis colorectal cancer. *Cancer Res.* **58** (1998), pp. 997–1003. Abstract-MEDLINE | Abstract-EMBASE

81. K. Okamoto, S. Toyokuni, K. Uchida, O. Ogawa, J. Takenewa, Y. Kakehi, H. Kinoshita, Y. Hattori-Nakakuki, H.H. Hiai and O. Hoshida, Formation of 8-hydroxy-2′-deoxyguanosine and 4-hydroxy-2-nonenal-modified proteins in human renal-cell carcinoma. *Int. J. Cancer* **58** (1994), pp. 825–829. Abstract-MEDLINE | Abstract-Elsevier BIOBASE | Abstract-EMBASE

82. M. Torzewski, M. Sarbia, H. Heep, P. Dutkowski, R. Willers and H.E. Gabbert, Expression of Bcl-X(L), an antiapoptotic member of the Bcl-2 family, in esophageal squamous cell carcinoma. *Clin. Cancer Res.* **4** (1998), pp. 577–583. Abstract-MEDLINE | Abstract-EMBASE

83. R.B. Kiningham, Physical activity and the primary prevention of cancer. *Prim. Care* **25** (1998), pp. 515–536. Abstract-MEDLINE | Abstract-EMBASE

84. J.L. Shisler, T.G. Senkevich, M.J. Berry and B. Moss, Ultraviolet-induced cell death blocked by a selenoprotein from a human dermatotropic poxvirus. *Science* **279** (1998), pp. 40–41.

85. N.G. Westman and S.L. Marklund, Copper- and zinc-containing superoxide dismutase and manganese-containing superoxide dismutase in human tissues and human malignant tumors. *Cancer Res.* **41** (1981), pp. 2962–2966. Abstract-EMBASE | Abstract-MEDLINE

86. D. Psathakis, N. Wedemeyer, E. Oevermann, F. Krug, C.P. Siegers and H.P. Bruch, Blood selenium and glutathione peroxidase status in patients with colorectal cancer. *Dis. Colon Rectum* **41** (1998), pp. 328–335. Abstract-MEDLINE | Abstract-EMBASE

87. W.H. Bannister and J.V. Bannister, Factor analysis of the activities of superoxide dismutase, catalase and glutathione peroxidase in normal tissues and neoplastic cell lines. *Free Radic. Res. Commun.* **41** (1987), pp. 1–13. Abstract-MEDLINE

88. Y. Li, A. Severn, M.V. Rogers, R.M. Palmer, S. Moncada and F.Y. Liew, Catalase inhibits nitric oxide synthesis and the killing of intracellular Leishmania major in murine macrophages. *Eur. J. Immunol.* **22** (1992), pp. 441–446. Abstract-MEDLINE | Abstract-EMBASE

89. J.F. Di Stefano, G. Beck and S. Zucker, Mechanism of BCG-activated macrophage-induced tumor cell cytotoxicity: evidence for both oxygen-dependent and independent mechanisms. *Int. Arch. Allergy Appl. Immunol.* **70** (1983), pp. 252–260. Abstract-EMBASE | Abstract-MEDLINE

90. M. Sundaresan, Z.X. Yu, V.J. Ferrans, K. Irani and T. Finkel, Requeriment for generation of H_2O_2 for platelet-derived-growth factor signal transduction. *Science* **270** (1995), pp. 296–299. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

91. T. Joneson and D. Bar-Sagi, A Rac1 effector site controlling mitogenesis through superoxide production. *J. Biol. Chem.* **273** (1998), pp. 17991–17994. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

92. A.M. Choi, K. Knobil, S.L. Otterbein, D.A. Eastman and D.B. Jacoby, Oxidant stress responses in influenza virus pneumonia: gene expression and transcription factor activation. *Am. J. Physiol.* **271** (1996), pp. L383–L391. Abstract-Elsevier BIOBASE | Abstract-MEDLINE

93. X. Wang, J.L. Martindale, Y. Liu and N.J. Holbrook, The cellular response to oxidative stress: influences of mitogen-activated protein kinase signalling pathways on cell survival.

Biochem. J. **333** (1998), pp. 291–300. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

94. F. Chen, Y. Lu, L.M. Demers, Y. Rojanasakul, X. Shi, V. Vallyathan and V. Castranova, Role of hydroxyl radical in silica-induced NF*kappa*B activation in macrophages. *Ann. Clin. Lab. Sci.* **28** (1998), pp. 1–13. Abstract-MEDLINE | Abstract-EMBASE

95. K.Y. Chia, S.P. Lim, A.A. Oei, T.K. Sabapathy and K.M. Hui, Acquisition of immunogenicity by AKR leukemic cells following DNA-mediated gene transfer is associated with the reduction of constitutive reactive superoxide radicals. *Int. J. Cancer* **57** (1994), pp. 216–223. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE

96. D. Offen, P.M. Beart, N.S. Cheung, C.J. Pascoe, A. Hochman, S. Gorodin, E. Melamed, R. Bernard and O. Bernard, Transgenic mice expressing human Bcl-2 in their neurons are resistant to 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine neurotoxicity. *Proc. Natl. Acad. Sci.* **95** (1998), pp. 5789–5794. Abstract-Elsevier BIOBASE | Abstract-MEDLINE | Abstract-EMBASE | **Full Text** via CrossRef

97. K.K. Kiningham and D.K.S. Clair, Overexpression of manganese superoxide dismutase selectively modulates the activity of Jun-associated transcription factors in fibrosarcoma cells. *Cancer Res.* **57** (1997), pp. 5265–5271. Abstract-EMBASE | Abstract-MEDLINE

98. C.K. Sen and L. Packer, Antioxidant and redox regulation of gene transcription. *FASEB J.* **10** (1996), pp. 709–720. Abstract-MEDLINE | Abstract-EMBASE

99. K.N. Desai, H. Wei and C.A. Lamartiniere, The preventive and therapeutic potential of the squalene-containing compound, Roidex, on tumor promotion and regression. *Cancer Lett.* **101** (1996), pp. 93–96. SummaryPlus | **Full Text + Links** | PDF (322 K)

100. K. Frenkel, Carcinogen-mediated oxidant formation and oxidative DNA damage. *Pharmacol. Ther.* **53** (1992), pp. 127–166. Abstract

---

Corresponding author. Tel.: +34-95-213-7135; fax: +34-95-213-2000; email: jmates@uma.es

The International Journal of Biochemistry & Cell Biology
Volume 32, Issue 2 , February 2000, Pages 157-170

```
Set      Items   Description
S1       32423   (MAGNET? OR ELECTROMAGNET? OR PARAMAGNET? OR FERROMAGNET?)-
             (3N)(FIELD? OR SPHERE? OR AREA? OR REGION? OR ZONE? OR PENUMB-
             R?)
S2       66363   MRI OR MAGNET?()RESONAN?
S3      628751   CANCER? OR TUMOR? OR TUMOUR? OR MALIGNAN? OR NEOPLAS? OR N-
             EO()PLAS? OR CARCINO?
S4       17362   FREE()RADICAL? OR OXYGEN()RADICAL?
S5         151   ATOM?(5N)(UNPAIR? OR UNMATCH? OR LOST)(2N)ELECTRON? ?
S6       47070   APOPTO? OR NECROSIS? OR NECROTI? OR NECROBIO? OR TUMORCID?
             OR TUMOURCID?
S7       36046   (CELL? ? OR CELLULAR?)(2N)(DEATH? OR DIE OR DIES OR DIED OR
              DYING OR KILL? OR SUICID?)
S8       39651   (THERAPY? OR THERAPEUT? OR THERAPIE? OR TREATMENT?)(3N)(RA-
             DIAT? OR GAMMA? OR GAMMARAY? OR SOUND? OR ACOUSTIC? OR ULTRAS-
             OUND? OR ULTRASON? OR ULTRA()(SONIC? OR SOUND?) OR PHOTO? OR -
             XRAY? OR X()(RAY OR RAYS OR RAYING OR RAYED))
S9     1383180   METHOD? ?
S10    8356011   SYSTEM? ?
S11    4060560   PROCESS??
S12    1620553   PROCEDURE? ?
S13    1017491   TECHNIQUE? ?
S14         54   S1 AND S3 AND S4:S5
S15         22   S14 AND S6:S7
S16          8   S14 AND S8
S17         51   S14 AND S9:S13
S18         24   S14 AND S2
S19         54   S14:S18
S20         47   S19 AND PY<2002
S21         39   RD (unique items)
? show files
File  16:Gale Group PROMT(R) 1990-2004/Jun 15
         (c) 2004 The Gale Group
File 160:Gale Group PROMT(R) 1972-1989
         (c) 1999 The Gale Group
File 148:Gale Group Trade & Industry DB 1976-2004/Jun 15
         (c)2004 The Gale Group
File 149:TGG Health&Wellness DB(SM) 1976-2004/Jun W1
         (c) 2004 The Gale Group
File 621:Gale Group New Prod.Annou.(R) 1985-2004/Jun 15
         (c) 2004 The Gale Group
File 444:New England Journal of Med. 1985-2004/Jun W2
         (c) 2004 Mass. Med. Soc.
File 441:ESPICOM Pharm&Med DEVICE NEWS 2004/Jun W2
         (c) 2004 ESPICOM Bus.Intell.
File 369:New Scientist 1994-2004/Jun W1
         (c) 2004 Reed Business Information Ltd.
File 370:Science 1996-1999/Jul W3
         (c) 1999 AAAS
File 129:PHIND(Archival) 1980-2004/Jun W1
         (c) 2004 PJB Publications, Ltd.
File 130:PHIND(Daily & Current) 2004/Jun 15
         (c) 2004 PJB Publications,Ltd.
File 135:NewsRx Weekly Reports 1995-2004/Jun W1
         (c) 2004 NewsRx
File  98:General Sci Abs/Full-Text 1984-2004/Jun
         (c) 2004 The HW Wilson Co.
File  15:ABI/Inform(R) 1971-2004/Jun 14
         (c) 2004 ProQuest Info&Learning
?
```

21/3,K/6 (Item 2 from file: 160)
DIALOG(R)File 160:Gale Group PROMT(R)

01803755
A NEW-OLD TOOL FOR STUDYING AGING, TISSUE DAMAGE AND CANCER
PR Newswire November 5, 1987 p. 1

A NEW-OLD TOOL FOR STUDYING AGING, TISSUE DAMAGE AND CANCER
Publication Year: **1987**

... unfold within intact, living organisms. In work described here today, they are adapting an existing **technique** called electron spin resonance spectroscopy, or ESR, to provide new information about aging, **cancer** and tissue damage, such as that which follows a heart attack. Referred to as in-vivo ESR, the new **technique** focuses on **free radicals** or highly reactive chemical species thought to be involved in a variety of biological mischief. **Free radicals** may be atoms or groups of **atoms** , but they always have an **unpaired electron** . Normally, **electrons** pair up, and their opposing spins and **magnetic fields** cancel each other. **Free radicals** have a detectable **magnetic field** that ESR picks up.
Full text available on PTS New Product Announcements. ...

PRODUCT NAME: Medical Imaging & Scanning; **Cancer** Diagnosis
EVENT NAME: Product Design & Development; Manufacturing **Processes**

21/3,K/11 (Item 5 from file: 148)
DIALOG(R)File 148:Gale Group Trade & Industry DB

01763530 SUPPLIER NUMBER: 02620495 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Treating cancer **ith heat.**
Lerch, Irving A.
Technology Review, v86, p14(5)
Feb-March, 1983
CODEN: TEREA ISSN: 0040-1692 LANGUAGE: ENGLISH RECORD TYPE: FULLTEXT
WORD COUNT: 2696 LINE COUNT: 00216

Treating cancer **ith heat.**

TEXT:
Treating **Cancer** with Heat
... medical research centers around the world have been experimenting with hyperthermia, a new form of **cancer** therapy that uses heat to manipulate the precarious balance of life. Hyperthermia alters the normal physiological environment of **tumor** cells, destroying their capacity to survive. But while heat produces devastating structural changes in **tumors** , the damage is slight or nonexistent in nearby healthy tissues. For reasons not completely understood, **tumors** seem to be more sensitive than normal tissue to the killing effects of heat.
This...

...of fever in healing disease. Busch himself was astonished when a woman with a facial **tumor** spontaneously recovered after contracting a feverish infection. By the early twentieth century, an American doctor...

...powerful adjunct to standard treatments rather than as a miracle therapy or "magic bullet' against **cancer** .
Researchers have found that heat therapy improves the effectiveness of other forms of **treatment** such as **radiation** . While there is some improvement with heat alone, the combined **treatment** of **radiation** and heat **therapy** controls progression of the disease in 25 to 50 percent of the patients treated. This...

...Armageddon
A small change in temperature can make a remarkable difference in the life or **death** of a **cell** . For example, cultures of the ovary cells of a Chinese hamster--a favorite target of...

...are exposed to 42.5 degrees centigrade for periods of increasing duration, the rate of **cell** killing is initially constant: each succeeding exposure destroys the same percentage of living cells--roughly 90...

...of glucose to form lactic acid. However, many cells do not experience this effect.
Within **tumors** , it appears that blood circulation is generally more sluggish than in normal healthy tissue. While...

...flush out excess heat and cool the cells down, blood slows down in heat-treated **tumor** cells. As the blood flow in the **tumor** continues to decline, vital nutrients and oxygen are with-held from the tissues until they begin to die and break apart. Some scientists believe that fragments from **dying tumor cells** may escape into the blood stream and stimulate the immune response that leads to hyperthermia cures. There have been

unconfirmed reports that advanced **cancers** have vanished in patients who received treatment at only one disease site.

Cellular studies also...

...responsible for molecular biosynthesis and cell reproduction.

While it is not clear exactly which biochemical **processes** heat therapy affects, researchers do agree that variations in cellular pH, oxygen concentration, and glucose...

...factors in treated tissues, it may be possible to protect normal cells while sensitizing the **tumor** to treatment.

The synergistic ...are more sensitive to radiation because the radiation reacts with water molecules to produce energetic **free radicals** (atoms energized with an extra electron). And these, in the presence of oxygen, produce poisons...

...for survival. The failure of some radiotherapy is attributed to the fact that many solid **tumors** outgrow their blood supply, making them oxygen-deficient--or hypoxic--at their peripheries and thus...

...when hyperthermia is combined with other treatments such as radiosensitizing drugs and chemotherapeutic agents.

Of **Tumors** , Mice, and Men

As the temperature is raised in animal **tumor systems** , researchers report an initial increase and then a rapid decrease in respiration. They detect a marked response when the **tumors** are heated to 42 to 43 degrees centigrade that is surprisingly similar to the results obtained with cell cultures. While the **tumor** may continue to grow for a few weeks after treatment, the rate of growth is less than that of the untreated control **tumors** . The **tumor** then begins to shrink and usually vanishes about five weeks after heat is applied. Interestingly, **tumors** are less apt to survive if the heat is applied locally rather than over the...

...treatment. While early results with whole-body hyperthermia were conflicting, recent reports from the Mississippi **Cancer** Center in Jackson indicate some room for optimism.

Part of the renewed interest in heat as a **cancer** treatment is due to advances in technology that have furnished the medical community with a ...

...a target temperature and maintained at that temperature for a specific time interval. And the **method** must not cause undue discomfort to the patient during or after therapy.

One of the...

...and the current follows the path defined by the electric field.

A difficulty with this **method** is that the current traces a path of least resistance and often fails to uniformly...

...coil made of a few turns of copper tubing. The coil produces alternating electric and **magnetic fields** in the exposed tissue. Superficial heating of skin and underlying layers is due to the electric component of the field. Deeper heating is induced by eddy currents generated by the **magnetic** component of the **field** . The larger the coil, the deeper the electric field penetrates. In this case, a single...

...to move the coil during therapy or to design multiple electrodes. One bonus of the **technique** is that magnetic material that couples with the coil's **electromagnetic field** may be implanted in the treated tissue in the form of needles or seeds. The...

...mixtures of ferromagnetic material. If successful, radiofrequency induction heating of such doped tissues could eradicate **tumors** in single treatments with little injury to nearby tissue.

Microwaves have also been used and found very effective in heating "shallow' **tumors** --those near the surface of the skin. Unfortunately, microwaves deposit a tremendous amount of energy...power levels. It appears that high-energy sound waves may be focused on a deep **tumor** , such as one in the prostate or cervix, with greater ease and accuracy than any...

...include uneven heating in the bone and loss of energy in the air, making the **method** unsuitable for heating lungs or gas-filled bowels. And some patients experience pain when bone...

...treatment because bone, being a good conductor of sonic waves, absorbs excess energy.

The hyperthermia **techniques** now available all have flaws. But technological advances should eventually provide us with more consistently successful **methods** . Even now, hyperthermia has the potential of helping the approximately 200,000 patients who fail to respond adequately to **radiation treatment** each year. This means that as many as 50,000 patients or more could be...

...which converts electrical energy into sonic waves. These high-powered

Photo: sound waves can heat **tumors** deep within the body with greater ease and accuracy than other hyperthermia **techniques** .

Photo: Above: The beneficial effect of heat, as interpreted by photographer Thomas Norton in an...

...body to 107 degrees Fahrenheit. Since human tissues are relatively poor conductors of heat, this **method** is not as effective in killing **tumors** as newer electronic **techniques** .

Photo: Below: Microwave heating works best against **tumors** on the surface of the skin or just below. Here a goldplated ceramic "horn' is...

...site of disease.

Photo: Above: Magnified 400 times in this photomicrograph, this densely packed human **tumor** thrives on nutrients supplied by the large blood vessel (the solid white part of the photo) that runs through its center. After hyperthermia treatment, the same **tumor** (top) is **dying** , its **cells** a mass of scarred tissue and its once healthy blood vessel shriveled and blocked off.

Photo: Why **cancer** cells are more sensitive to heat than normal cells is still a matter of conjecture...

...illustrated in this diagram, is that the vasculature (the structural web of blood vessels) inside **tumor** cells is not as efficient as the vasculature inside normal cells. When a normal cell...

...increasing blood flow and flushing out the excess heat. But the intertwined vessels in a **tumor** cell are unable to get rid of heat in this manner and blood flow slows down. As a result, the **tumor** cell is deprived of vital nutrients and eventually dies.

...DESCRIPTORS: **Cancer** --
19830200

Summary of abstracts
22nd International Clinical Hyperthermia Society

September 23, 1999
Marina Del Rey, Los Angeles, CA, USA.

(Alphabetical order, by authors)

THERMOREGULATORY MECHANISM IN THE MALIGNANT CARCINOMA TREATMENT BY ELECTROMAGNETIC HYPERTHERMIA COMBINED WITH RADIOCHEMOTHERAPY

Mykhalkin, I., M. D., Ph. D.

National Analytical Center Medium, Institute of Oncology; Kiev, Ukraine

Electromagnetic hyperthermia with radiation therapy and chemotherapy were carried out in 165 patients with malignant tumors. Analogous experimental investigations were accomplished on mice, rats and dogs. Rehabilitation of patients was accelerated, rate of irradiation reaction was dimensioned and terms of treatment of the patients were shortened. In addition the delay of transplanted tumors growth and the increasing of lifetime of animals were achieved. Various methods of electromagnetic hyperthermia make quite different structures of heat productions of heterogeneous tissue. Microwave heating supplies the zone of effective heat production in muscular tissue as deep as 2-4cm., with maximum of the heat production on the surface of skin.

Usage of strong heating makes the patients to feel pain. Application of coolers are widely used in electromagnetic hyperthermia. The heat receptors do not react with pain. Our cancer patients sensed some discomfort, the feeling of pressure in the tissue or could not express their feeling under electromagnetic hyperthermia.

The temperature in the tumor may go as high as 47 degree Celsius. The muscular tissue temperature goes up to 41-43 degree Celsius because of intensive blood circulation. The use of in inductive heating with the help of changing magnetic field gives the maximum heating influence to the tissue obtaining much liquid. That's why the implementation of the inductive method is very effective in case of the tumors of soft tissue. Together with the mediLAK Company we worked out inductive irradiators with low level of heating. They undergo some approbation in oncological and orthopedy clinics in Kyiv and analongs of our irradiators from 1993 in Japan. Such irradiators are especially effective when used to treat different inflammation diseases.

Journal of Photochemistry and Photobiology B: Biology
Volume 64, Issue 1 , 1 November 2001, Pages 21-26

doi:10.1016/S1011-1344(01)00185-3 Cite or Link Using DOI

Photodynamic effect on cancer cells influenced by electromagnetic fields

Lijun Pang[a], Cristina Baciu[b], Nelly Traitcheva[c] and Hermann Berg[d]

[a] Institute of Physics, Nankai University, Nankai, PR China
[b] Physics Department, University of Ploiesti, Ploiesti, Romania
[c] Institute of Plant Physiology 'M. Popov', Bulgarian Academy of Sciences, Sofia, Bulgaria
[d] Laboratory Bioelectrochemistry, Jena, Saxonian Academy of Sciences, Leipzig, Germany

Received 4 May 2001; accepted 7 August 2001. Available online 6 November 2001.

Abstract

The synergism of low-frequency electromagnetic field treatment and photodynamic effect on killing of human cancer cells is presented. The weak pulsating electromagnetic field (PEMF) generated by Helmholtz coils in the mT range influences the permeability of cell membranes for photosensitizers. Several types of sensitizers were excited by visible light during incorporation without and with two kinds of PEMF treatment. In the first part suitable

photosensitizers were selected in the absorption range between 400 and 700 nm against human myeloid leukaemia K562 and human histiocytic lymphoma U937 cells by treatment of PEMF consisting of rectangular pulse groups. In the second part amplitude and frequency dependencies were measured using sinuous PEMF and white light with the result that after 12 min the PEMF treatment enhanced photodynamic effectivity by more than 40% over the control value. Taking into account the influence of many parameters, an additional optimization will be possible by photodynamic PEMF synergism for an increased drug delivery in general.

Author Keywords: Cancer cells; Photodynamic effect; Electromagnetic field; Synergism

```
Set     Items   Description
S1     956411   (MAGNET? OR ELECTROMAGNET? OR PARAMAGNET? OR FERROMAGNET?)-
             (3N)(FIELD? OR SPHERE? OR AREA? OR REGION? OR ZONE? OR PENUMB-
             R?)
S2    1192532   MRI OR MAGNET?()RESONAN?
S3    7362474   CANCER? OR TUMOR? OR TUMOUR? OR MALIGNAN? OR NEOPLAS? OR N-
             EO()PLAS? OR CARCINO?
S4     327861   FREE()RADICAL? OR OXYGEN()RADICAL?
S5        754   ATOM?(5N)(UNPAIR? OR UNMATCH? OR LOST)(2N)ELECTRON? ?
S6    1249301   APOPTO? OR NECROSIS? OR NECROTI? OR NECROBIO? OR TUMORCID?
             OR TUMOURCID?
S7     526766   (CELL? ? OR CELLULAR?)(2N)(DEATH? OR DIE OR DIES OR DIED OR
              DYING OR KILL? OR SUICID?)
S8     375319   (THERAPY? OR THERAPEUT? OR THERAPIE? OR TREATMENT?)(3N)(RA-
             DIAT? OR GAMMA? OR GAMMARAY? OR SOUND? OR ACOUSTIC? OR ULTRAS-
             OUND? OR ULTRASON? OR ULTRA()(SONIC? OR SOUND?) OR PHOTO? OR -
             XRAY? OR X()(RAY OR RAYS OR RAYING OR RAYED))
S9   17109240   METHOD? ?
S10  25513614   SYSTEM? ?
S11   7711606   PROCESS??
S12   3219660   PROCEDURE? ?
S13   8956709   TECHNIQUE? ?
S14       105   S1 AND S3 AND S4:S5
S15        10   S14 AND S6:S7
S16         2   S14 AND S8
S17        74   S14 AND S9:S13
S18        15   S14 AND S2
S19       105   S14:S18
S20        86   S19 AND PY<2002
S21        58   RD (unique items)
? show files
File 155:MEDLINE(R) 1966-2004/Jun W1
         (c) format only 2004 The Dialog Corp.
File   2:INSPEC 1969-2004/Jun W1
         (c) 2004 Institution of Electrical Engineers
File   5:Biosis Previews(R) 1969-2004/Jun W1
         (c) 2004 BIOSIS
File   6:NTIS 1964-2004/Jun W2
         (c) 2004 NTIS, Intl Cpyrght All Rights Res
File   8:Ei Compendex(R) 1970-2004/Jun W1
         (c) 2004 Elsevier Eng.  Info. Inc.
File  34:SciSearch(R) Cited Ref Sci 1990-2004/Jun W1
         (c) 2004 Inst for Sci Info
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
         (c) 1998 Inst for Sci Info
File  73:EMBASE 1974-2004/Jun W1
         (c) 2004 Elsevier Science B.V.
File  71:ELSEVIER BIOBASE 1994-2004/Jun W1
         (c) 2004  Elsevier Science B.V.
File 144:Pascal 1973-2004/Jun W1
         (c) 2004 INIST/CNRS
File  35:Dissertation Abs Online 1861-2004/May
         (c) 2004 ProQuest Info&Learning
File  65:Inside Conferences 1993-2004/Jun W2
         (c) 2004 BLDSC all rts. reserv.
File  94:JICST-EPlus 1985-2004/May W4
         (c)2004 Japan Science and Tech Corp(JST)
File  95:TEME-Technology & Management 1989-2004/May W4
         (c) 2004 FIZ TECHNIK
File  99:Wilson Appl. Sci & Tech Abs 1983-2004/May
         (c) 2004 The HW Wilson Co.
```

File 481:DELPHES Eur Bus 95-2004/May W5

File 583:Gale Group Globalbase(TM) 1986-2002/Dec 13

?

21/3,K/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10178289 PMID: 8066117
Model for the rationalization of magnetic field **effects in vivo. Application of the radical-pair mechanism to biological** systems .
Scaiano J C; Cozens F L; McLean J
Department of Chemistry, University of Ottawa, Canada.
Photochemistry and photobiology (UNITED STATES) Jun **1994**, 59 (6) p585-9, ISSN 0031-8655 Journal Code: 0376425
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Model for the rationalization of magnetic field **effects in vivo. Application of the radical-pair mechanism to biological** systems .
Jun **1994** ,
A model for **magnetic field** effects in biological **systems** is proposed. This model employs the basic concepts of the radical pair mechanism, and predicts that **magnetic fields** will increase the average radical concentration, lengthen their lifetime and enhance the probability of radical reactions with cellular components. The relevance of these effects in relation to **cancer** initiation, promotion and progression is discussed.
; **Free Radicals** ; **Neoplasms** --etiology--ET
Chemical Name: **Free Radicals**

21/3,K/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05277797 PMID: 6892743

Enhanced antitumor effect in the combined action of a magnetic field **and hypothermia]**

Usilenie protivoopukholevogo effekta pri kombinirovannom deistvii magnitnogo polia i gipotermii.

Lu B N; Iakupova R M; Kauashev S K

Voprosy onkologii (USSR) **1980** , 26 (3) p55-9, ISSN 0507-3758
Journal Code: 0413775
Document type: Journal Article ; English Abstract
Languages: RUSSIAN
Main Citation Owner: NLM
Record type: Completed

Enhanced antitumor effect in the combined action of a magnetic field **and hypothermia]**

1980 ,

To substantiate some concepts of an oxygen-peroxide model of **carcinogenesis** , a number of experiments were conducted, and also the data were utilized previously obtained by the writers on the directed flow of water dissolved oxygen influenced by a permanent **magnetic field** . The observed suppressive action of the **magnetic field** on the growth of transplantable Pliss lymphosarcoma and PC-1 **tumor** may be accounted for the latter uncoupling oxygen in actively growing hyperoxic **neoplastic** cells from other participants of the direct **free radical** oxidation, spliting or minimizing in them closed cycles of reproduction of toxic products of lipids...

Descriptors: Hypothermia, Induced; *Magnetics; * **Neoplasms** , Experimental --therapy--TH; Animals; Lymphoma, Non-Hodgkin--therapy--TH; **Neoplasm** Transplantation; Rats; Time Factors

21/3,K/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

02941490 PMID: 5384579
Influence of a constant magnetic field **on the ascitic** tumor **sarcoma** 37]
Deistvie postoiannogo magnitnogo polia na astsitnuiu opukhol' sarkomu 37.
Piruzian L A; Markuze I I; Chibrikin V M
Izvestiia Akademii nauk SSSR. Seriia biologicheskaia (USSR) Nov-Dec **1969**, 6 p893-8, ISSN 0002-3329 Journal Code: 7505543
Document type: Journal Article
Languages: RUSSIAN
Main Citation Owner: NLM
Record type: Completed

Influence of a constant magnetic field **on the ascitic** tumor **sarcoma** 37]
Nov-Dec **1969** ,
; Animals; **Free Radicals** ; Mice
Chemical Name: **Free Radicals**

21/3,K/20 (Item 1 from file: 2)
DIALOG(R)File 2:INSPEC

6935721 INSPEC Abstract Number: A2001-13-8760D-006
Title: Lysis of photosensitized erythrocytes in an alternating magnetic field
Author(s): Babincova, M.; Leszczynska, D.; Sourivong, P.; Babinec, P.
Author Affiliation: Dept. of Biophys. & Chem. Phys., Comenius Univ., Bratislava, Slovakia
Journal: Journal of Magnetism and Magnetic Materials Conference Title: J. Magn. Magn. Mater. (Netherlands) vol.225, no.1-2 p.194-6
Publisher: Elsevier,
Publication Date: April 2001 Country of Publication: Netherlands
CODEN: JMMMDC ISSN: 0304-8853
SICI: 0304-8853(200104)225:1/2L.194:LPEA;1-9
Material Identity Number: J271-2001-010
U.S. Copyright Clearance Center Code: 0304-8853/2001/$20.00
Conference Title: Third International Conference on Scientific and Clinical Applications of Magnetic Carriers
Conference Date: 3-6 May 2000 Conference Location: Rostock, Germany
Language: English
Subfile: A

Title: Lysis of photosensitized erythrocytes in an alternating magnetic field
Abstract: Exposure of human erythrocytes photosensitized with hematoporphyrin dihydrochloride to a **magnetic field** (180 kHz, 4.6 kA/m) resulted in time- and hematoporphyrin-concentration-dependent lysis. The...

...of radicals in erythrocyte lysis. Exposure not only to light but also to an AC **magnetic field** may thus enhance the efficiency of **photodynamic tumor therapy** .
...Descriptors: **free radical** reactions...

... **photodynamic therapy**
...Identifiers: alternating **magnetic field** ; ...

... **photodynamic tumor therapy** ; ...

... **cancer** treatment
2001

21/3,K/21 (Item 2 from file: 2)
DIALOG(R)File 2:INSPEC

6553935 INSPEC Abstract Number: A2000-10-8750-001
Title: Physics may help chemistry to improve medicine: a possible mechanism for anticancer activity of static and ELF magnetic fields
Author(s): Tofani, S.
Author Affiliation: Servizio di Fisica Sanitaria, Ivrea Hosp., Italy
Journal: Physica Medica vol.15, no.4 p.291-4
Publisher: Istituti Editoriali e Poligrafici Internazionali,
Publication Date: Oct.-Dec. 1999 Country of Publication: Italy
CODEN: PHYME2 ISSN: 1120-1797
SICI: 1120-1797(199910/12)15:4L.291:PHCI;1-T
Material Identity Number: G240-2000-001
Language: English
Subfile: A

...**Title: help chemistry to improve medicine: a possible mechanism for anticancer activity of static and ELF** magnetic fields
Abstract: A biophysical model explaining how static and Extremely Low Frequency (ELF) **magnetic fields** of more than 1 mT may selectively interfere in cell survival **processes** of pathological cells (i.e., **cancer** cells) is reported. This model considers the hyperfine interaction mechanism on electron spin phase coherence influencing **free radical** recombination, redox signaling and corresponding cell signaling mediated events. The different electrical behavior of pathological...

... and normal ones, is considered the cause of the selective response of transformed cells to **magnetic fields** . Thus, new frontiers in the treatment of diseases characterized by an alteration of cell survival...
...Descriptors: **cancer** ; ...

... **free radicals** ; ...

... **magnetic field** effects...

... **radiation therapy**
...Identifiers: ELF **magnetic fields** ; ...

...static **magnetic fields** ; ...

...cell survival **processes** interference...

... **free radical** recombination
1999

21/3,K/23 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0010744824 BIOSIS NO.: 199799378884
A 3 milliTesla 60 Hz magnetic field **is neither mutagenic nor Co-mutagenic in the presence of menadione and MNU in a transgenic rat cell line**
AUTHOR: Suri Andrew; Deboer Johan; Kusser Wolfgang; Glickman Barry W (Reprint)
AUTHOR ADDRESS: Cent. Environ. Health, Dep. Biol., Univ. Victoria, P.O. Box 3020, Victoria, BC V8W 3N5, Canada**Canada
JOURNAL: Mutation Research 372 (1): p23-31 1996 **1996**
ISSN: 0027-5107
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

A 3 milliTesla 60 Hz magnetic field **is neither mutagenic nor Co-mutagenic in the presence of menadione and MNU in a...**
1996

ABSTRACT: The mechanisms by which an **electromagnetic field** (EMF) influences biological material are poorly understood. One potentially important model suggests that a **magnetic field** can stabilize **free radicals** in such a way as to permit their dispersement rather than their return to the...

...fibroblast cell line, R2-lambda-LIZ. Mutant frequencies were determined in cells exposed to a **magnetic field** , cells pretreated with the mutagens N-methylnitrosourea (MNU) or 2-methyl-1,4-naphthoquinone (menadione), prior to being held in a 60 Hz 3 milliTesla (mT) **magnetic field** and cells concurrently exposed to the mutagens and the **magnetic field** . Menadione was selected because its mutagenic mechanism involves the formation of **free radicals** , while MNU is an alkylating agent not thought to act through radical formation. According to the radical stabilization hypothesis the application of a **magnetic field** to menadione treated cells would accentuate the mutagenic effects. Our results failed to indicate that the **magnetic field** affects mutagenesis by the **oxygen - radical** mediated mutagen, menadione.
DESCRIPTORS:
...MAJOR CONCEPTS: **Tumor** Biology
MISCELLANEOUS TERMS: ... **ELECTROMAGNETIC FIELD** ;

21/3,K/24 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0002423334 BIOSIS NO.: 197866009818
THE EFFECT OF A CONSTANT MAGNETIC FIELD **ON VARIOUS MODELS OF** CARCINOGENESIS
AUTHOR: KOGAN A KH (Reprint); KULITSKAYA V I
AUTHOR ADDRESS: DIV PATHOL PHYSIOL, IM SECHENOV FIRST MOSC MED INST, MOSCOW, USSR**USSR
JOURNAL: Patologicheskaya Fiziologiya i Eksperimental'naya Terapiya (2): p 63-68 **1977**
ISSN: 0031-2991
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: RUSSIAN

THE EFFECT OF A CONSTANT MAGNETIC FIELD **ON VARIOUS MODELS OF** CARCINOGENESIS
1977

ABSTRACT: Experiments were done in 3330 rats. A study was made of the effect on **carcinogenesis** of a constant **magnetic field** (CMF). It appeared that under its influence **carcinogenesis** displayed ordinary stages, but had a number of differences. Sarcoma growth was accelerated. There was...

...the latent period of development. The incidence of less differentiated (polymorphocellular) sarcomas was increased. Peroxide **free - radical** lipid oxidation was potentiated at all stages of the pretumor period. The mechanism of the effect of the CMF on **carcinogenesis** is discussed.
DESCRIPTORS: RAT SARCOMA PER OXIDE **FREE RADICAL** LIPID OXIDATION/
DESCRIPTORS:
...MAJOR CONCEPTS: Skeletal **System** --...

... **Tumor** Biology

21/3,K/31 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

!!

09942274 Genuine Article#: 468FJ No. References: 20
Title: Static and ELF magnetic fields **induce** tumor **growth inhibition and** apoptosis
Author(s): Tofani S (REPRINT) ; Barone D; Cintorino M; de Santi MM; Ferrara A; Orlassino R; Ossola P; Peroglio F; Rolfo K; Ronchetto F
Corporate Source: Ivrea Hosp,Dept Med Phys, ASL 9,Via Di Vittorio 1/I-10015 Ivrea/TO/Italy/ (REPRINT); Ivrea Hosp,Dept Med Phys, ASL 9,I-10015 Ivrea/TO/Italy/; LCG BIOSCI RBM,Colleretto Giacosa/TO/Italy/; Univ Siena,Inst Pathol Anat & Histol,I-53100 Siena//Italy/
Journal: BIOELECTROMAGNETICS, **2001** , V22, N6 (SEP), P419-428
ISSN: 0197-8462 Publication date: 20010900
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

Title: Static and ELF magnetic fields **induce** tumor **growth inhibition and** apoptosis
, **2001**
Abstract: The ability of static and extremely low frequency (ELF) **Magnetic Fields** (MF) to interfere with **neoplastic** cell function has been evaluated. In vitro experiments were carried out to study the role...
...human breast adenocarcinoma) and one nontransformed cell line (MRC-5 embryonal lung fibroblast). Increase in **cell death** morphologically consistent with **apoptosis** was reported exclusively in the two transformed **cell** lines. **Cell - death** induction was observed with MF of more than 1 mT. It was independent of the...

...to treat nude mice xenografted with WiDr cells. The treatment of nude mice bearing WiDr **tumors** subcutaneously. with daily exposure for 70 min to MF for 4 weeks caused significant **tumor** growth inhibition (up to 50%) by the end of the treatment when modulated MF were...
...Identifiers--PULSED **ELECTROMAGNETIC - FIELDS** ; BLOOD MONONUCLEAR-CELLS; BIOLOGICAL- **SYSTEMS** ; **FREE - RADICALS** ; CYTOKINES; MECHANISM

21/3,K/44 (Item 15 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

03076494 Genuine Article#: NE168 No. References: 43
Title: POTENTIAL USE OF NITROXIDES IN RADIATION ONCOLOGY
Author(s): HAHN SM; KRISHNA CM; SAMUNI A; DEGRAFF W; CUSCELA DO; JOHNSTONE P; MITCHELL JB
Corporate Source: NCI,RADIAT ONCOL BRANCH,9000 ROCKVILLE PIKE,BLDG 10,ROOM B3B69/BETHESDA//MD/20892; NCI,RADIAT BIOL SECT/BETHESDA//MD/20892; HEBREW UNIV JERUSALEM,SCH MED/IL-91010 JERUSALEM//ISRAEL/
Journal: CANCER RESEARCH, **1994** , V54, N7 (APR 1), PS2006-S2010
ISSN: 0008-5472
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

, **1994**
...Abstract: in vivo radioprotectors, the nitroxides, has been discovered. The nitroxides are low-molecular-weight stable **free radicals** which are freely membrane permeable and which have been shown to act as superoxide dismutase...

...of reduced transition metals, superoxide dismutase-like activity, and scavenging of oxy- and carbon-based **free radicals** . In vivo studies reveal that Tempol protects C3H mice from the lethal effects of radiation...
...and toxicity as well as to fully evaluate the extent to which these compounds protect **tumors** .
...Research Fronts: WR-2721; AZACYTIDINE-TREATED XRS5 CELLS; TOXICITY PROTECTANTS)
92-3383 001 (MICELLIZED RADICAL PAIRS; EXTERNAL **MAGNETIC - FIELD** ; RECOMBINATION KINETICS; ELECTRON-SPIN POLARIZATION)
92-3689 001 (INTERSTITIAL HYPERTENSION IN SOLID **TUMORS** ; QUINONE METHIDE; HYPOXIC EMT6 CELLS; REDUCTION OF DAUNOMYCIN; INTRACELLULAR PH; MICROVASCULAR PRESSURE)
92-3867 001 (RIF-1 **TUMOR** ; RADIATION RESPONSE OF A C3H MOUSE MAMMARY-**CARCINOMA** ; P-31 **MAGNETIC - RESONANCE** SPECTROSCOPY; RENAL-FUNCTION IN MICE; BLOOD PERFUSION VOLUME)
92-7714 001 (RECOMBINANT HUMAN GRANULOCYTE COLONY...

25/3,K/73 (Item 73 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00280696 **Image available**
NOVEL THERAPEUTIC DELIVERY SYSTEMS
NOUVEAU SYSTEME **D'ADMINISTRATION DE PRODUITS THERAPEUTIQUES**
Patent Applicant/Assignee:
UNGER Evan C,
FRITZ Thomas A,
MATSUNAGA Terry,
RAMASWAMI VaradaRajan,
YELLOWHAIR David,
WU Guanli,
Inventor(s):
UNGER Evan C,
FRITZ Thomas A,
MATSUNAGA Terry,
RAMASWAMI VaradaRajan,
YELLOWHAIR David,
WU Guanli,
Patent and Priority Information (Country, Number, Date): =(US) 5580575
Patent: WO 9428874 A1 19941222
Application: WO 94US5633 19940519 (PCT/WO US9405633)
Priority Application: US 9376250 19930611; US 93159674 19931130; US 93159687 19931130; US 93160232 19931130
Designated States: AU CA CN JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 50080

NOVEL THERAPEUTIC DELIVERY SYSTEMS
NOUVEAU SYSTEME **D'ADMINISTRATION DE PRODUITS THERAPEUTIQUES**
Fulltext Availability:
Detailed Description
Claims

English Abstract
Therapeutic delivery **systems** comprising gaseous precursor-filled microspheres comprising a therapeutic are described. **Methods** for employing such microspheres in therapeutic delivery applications are also provided. Therapeutic delivery **systems** comprising gaseous precursor-filled liposomes having encapsulated therein a contrast agent or drug are preferred. **Methods** of and apparatus for preparing such liposomes and **methods** for employing such liposomes in therapeutic delivery applications are also disclosed.
French Abstract
Systeme d'administration de produits therapeutiques comportant des microspheres remplies d'un precurseur gazeux comportant un...

Detailed Description
NOVEL THERAPEUTIC DELIVERY **SYSTEMS**
RELATED APPLICATIONS
This application is a continuation-in-part of co pending applications U.S...

...entirety.

BACKGROUND OF THE INVENTION

Field of the Invention
This invention relates to therapeutic delivery **systems** , and more specifically, to gaseous precursorcontaining microspheres comprising a therapeutic compound.

The invention further relates to **methods** for employing such microspheres as therapeutic delivery **systems** .

Background of the Invention
Targeted therapeutic delivery means are particularly important where the toxicity of a drug is an issue. Specific therapeutic delivery **methods** potentially serve to minimize toxic side effects, lower the required dosage amounts, and decrease costs...

...and/or
other important needs in the area of therapeutic delivery.

A variety of imaging **techniques** have been used for detection and diagnosis of diseases in animals and humans.

X-rays represent one of the first **techniques** used for diagnostic imaging. The images obtained through this **technique** reflect the electron density of the object being imaged. Contrast agents, such as barium or...

...is ionizing, and the various
deleterious effects of ionizing radiation are cumulative.

Another important imaging **technique** is magnetic resonance imaging (MRI). This **technique** , however, has various drawbacks, such as expense and shear size of an MRI scanner...
...not available at many
medical centers.

Radionuclides, employed in nuclear medicine, provide a further imaging **technique** . In employing this **technique** , radionuclides such as technetium labeled compounds are injected into the patient, and images are obtained from gamma cameras. Nuclear medicine **techniques** , however, suffer from poor spatial resolution and expose the animal or patient to the deleterious...

...Furthermore, the
handling and disposal of radionuclides is problematic.

Ultrasound is another diagnostic imaging **technique** which is unlike nuclear medicine and X-rays since it does not expose the patient...

...is relatively inexpensive and may be conducted as a portable examination. In using the ultrasound **technique** , sound is transmitted into a patient ...are reflected by an interface they are detected by the receiver in the transducer and **processed** to form an image. The acoustic properties of the tissues and fluids within the body...

...based on gas
bubbles or gas containing bodies and on the development of

efficient **methods** for their preparation.

PCT/IJS94/05633
Ryan et al., in U-S- Patent 4,S44f54S...

...Patents 4,684,479 and S,21S,680,
teaches a gas-in-liquid emulsion and **method** for the
production thereof from surfactant mixtures. U.S. Patent
4,684,479 discloses the...

...medium in air. U.S.

Patent S,21S,680 is directed to a large scale **method** of
IS producing lipid coated microbubbles including shaking a
solution of the surfactant in liquid ...05633
widder, in U.S. Patents 4,572,203 and 4,844,882,
disclose a **method** of ultrasonic imaging and a microbubbletype
ultrasonic imaging agent.

Quay, in WO 93/05819, describes...

...substantial surface activity or
water solubility and a surfactant. Kaufman et al. also teach
a **method** of using the emulsion in medical applications.

Another area of significant research effort is in
the area of targeted drug delivery. The **methods** and
materials in the prior art for introduction of genetic
materials to, for example, living...

...mechanisms have been
20 developed to deliver genetic material to living cells. These
mechanisms include **techniques** such as calcium phosphate
precipitation and electroporation, and carriers such as
cationic polymers and aqueous-filled liposomes. These
methods have all been relatively ineffective in vivo and only
25 of limited use for cell culture transfection. None of these
methods potentiate local release, delivery and integration of
genetic material to the target cell.
Better means...

...genetic
PCT[US94/05633
material at the surface of selected cell membranes. A
variety of **techniques** have been tried in vivo but without
great success. For example, viruses such as adenoviruses vivo for
cellular delivery
of genetic material. For example, cationic liposome
20 transfection **techniques** have not worked effectively in vivo.

More effective means are needed to improve the cellular...

...and delivery of
genetic material.

SLWLzLRY OF THE INVENTION
The present invention provides therapeutic delivery
systems for site-specific delivery of therapeutics using gasfilled
microspheres. The microspheres contain a temperature.

activated...
...to rupture and release the therapeutic compound.

Specifically, the present invention provides targeted therapeutic delivery **systems** comprising a temperature activated gaseous precursor-filled microsphere comprising a therapeutic compound.

The invention also contemplates **methods** for the controlled delivery of therapeutic compounds to a region of a patient comprising: W...

...35 to release the therapeutic compound in the region.

In addition, the present invention provides **methods** - and apparatus for preparing temperature activated gaseous precursor-filled liposomes suitable for use in delivery of contrast agents and as drug delivery agents. Preferred 5 **methods** of the present invention provide the advantages, for example, of simplicity and potential cost savings...substantially devoid of water in the interior thereof prepared by the vacuum drying gas instillation **method** , without any drugs encapsulated therein. The data was obtained by scanning with a 7.5...

...Acoustic Imaging' Model 5200 scanner (Acoustic Imaging, Phoenix, Arizona), and was generated by using the **system** test software to measure reflectivity. The **system** was standardized prior to each experiment with a phantom of known acoustic impedance.

FIGURE 14...

...substantially devoid of water in the interior thereof prepared by the vacuum drying gas instillation **method** .

FIGURE 15 is a micrograph which shows the sizes of 35 gaseous precursor-filled liposomes...

...liposome diameter.

DETAILED DESCRIPTION OF THE INVENTION
The present invention provides a targeted therapeutic delivery **system** comprising a temperature activated gaseous precursor-filled microsphere comprising a therapeutic compound. A microsphere is...However, the gaseous precursor may be in liquid or gaseous phase for use in the **methods** of the present invention. Suitable temperature activated gaseous precursors are well known to those skilled...

...upon entering the patient or animal, prior to use, during storage, or during manufacture. The **methods** of producing the ...This embodiment is prepared by introducing the gaseous precursor to the microsphere during the manufacturing **process** .

The gaseous precursors may be utilized to create

stable gas-filled microspheres which are pre...

...state. In so doing,
the precursor converts to the gaseous state during the
15 microemulsification **process** . In the presence of the
appropriate stabilizing agents surprisingly stable gas-filled
liposomes result.
Similarly...5rewarming (e.g. injection in vivo) the appropriate sized gas
liposomes then form.

An alternate **method** of entrapping the
perfluoropentane gaseous precursor is illustrated. A small
quantity (0.76 - 1.54...

...is added to an
10 aqueous solution of lipids as described above, with a
different **method** of agitation utilized. The material is
placed in a Microfluidizer (Microfluidics, Newton, MA) and
subject...

...coated with lipid and the size of the
resultant liposomes is controlled. In a manufacturing
process , the unentrapped perfluoropentane could be removed by
25 several ways. Firstly, liquid perfluoropentane is dense...

...quickly to the top of a vessel and can thereby
be removed. A very practical **method** is to use filtration
which can be performed as an in-line **process** during injection
into the patient.
A micellular formulation may be substituted for a
liposome (lipidoptionally with a portion
of PEGylated lipids). A microfluidizer **process** can be used
to produce a micellular formulation of the gaseous precursor
5 to produce...

...in
vivo or designed to produce the gas-filled liposome in situ,
during the manufacturing **process** , on storage, or at some time
prior to use. Knowing the amount of liquid in...gaseous precursors
activated by temperature.

In fact, depression of the freezing point of the solvent
system is allows the use gaseous precursors which would
undergo liquid-to-gas phase transitions at temperatures below
is 00 C. The solvent **system** can be selected to provide a medium
for suspension of the gaseous precursor. For example...

...sodium chloride, the freezing point
can be depressed even further.

The selection of appropriate solvent **systems** may be
explained by physical **methods** as well. When substances,
solid or liquid, herein referred to as solutes, are dissolved
in...or solid solute
necessary to depress the solvent freezing temperature to an
25 appropriate value.

Methods of preparing the temperature activated
gaseous precursor-filled liposomes include.

vortexing an aqueous suspension of gaseous precursor-filled liposomes of the present invention; 30 variations on this **method** include optionally heating an aqueous suspension of gaseous precursor and lipid, optionally venting the vessel...

...resulting liposomes such that a filter of about 0.22 gm is employed; a microemulsification **method** whereby an aqueous suspension of gaseous precursor-filled liposomes of the present invention are emulsified prior to the shaking gas instillation **method** . Drying-gas instillation **method** may be used to remove water from liposomes. By pre-entrapping the gaseous precursor in...

...50 C.

The gaseous precursor-filled microspheres can be used in conjunction with such clinical **techniques** as ultrasound, microwave radiation, or electromagnetic energy to generate liquid to gas conversion of the...

...difficult to solubilize in aqueousbased formulation.

As one skilled in the art would recognize, this **process** of microemulsification, for example, gas-filled microsphere stabilization from temperature activated gaseous precursors, can be...

...produce a wide variety of improved stabilized gaseous microsphere products.

By selecting the appropriate solvent **system** and gaseous precursor, as well as stabilizing agents, microspheres improved over conventional products can be prepared. The solvent **system** can be selected to provide a ligand medium for suspension of the gaseous precursor. As...

...point can be depressed even further. Depression of 35 the freezing point of the solvent **system** is important in that this allows us to use gaseous precursors which would undergo liquid...diffusible and less soluble in aqueous media than air or nitrogen, the resultant therapeutic delivery **systems** generally more stable than traditional gas or non-precursor based contrast agents.

By "gas-filled...small liposome.

The lipids also serve to stabilize the resultant microsphere size. In this case, **techniques** such as microemulsification are preferred for forming the small liposomes which entrap the precursor. A...recirculation. The gaseous precursor-filled microspheres may be coated such that uptake by the reticuloendothelial **system** is minimized. Useful coatings include, for example, gangliosides, glucuronate, galacturonate, guluronate, polyethyleneglycol, polypropylene glycol, polyvinylpyrrolidone...

...The microspheres may also be coated for purposes such as evading recognition by the immune **system** .

is In preferred embodiments, at least about 50@0o, preferably, at least about 75@0o...and 5 mole percent dipalmitoylphosphatidic acid.

In addition, examples of compounds used to make.mixed **systems** include, but by no means are limited to lauryltrimethylammonium bromide (dodecyl-), - 38 cetyltrimethylammonium bromide (hexadecyl...

...the suspensions. A preferred product of the present invention incorporates lipid as a mixed solvent **system** in a ratio of 8:1:1 or ...or stabilizing agents are included with the gaseous precursors to formulate the therapeutic containing delivery **system** . The purpose of these emulsifying/stabilizing agents is two-fold. Firstly, these agents help to...of the above precursors may also be used to deliver antisense DNA or chemotherapeutics to **tumors** . It is postulated that subtle changes in temperature, pH, and oxygen tension are responsible for...different types of diseases. For example, adenosine deaminase may be provided to treat ADA deficiency; **tumor necrosis** factor and/or interleukin-2 may be provided to treat advanced **cancers** ; HDL receptor may be provided to treat liver disease; thymidine kinase may be provided to treat 20 ovarian **cancer** , brain **tumors** , or HIV infection; HLA-B7 may be provided to treat **malignant** melanoma; interleukin-2 may be provided to treat neuroblastoma, **malignant** melanoma, or kidney **cancer** ; interleukin-4 may be provided to treat **cancer** ; HIV env may be provided to treat HIV infection; antisense .

25 ras/p53 may be provided to treat lung **cancer** ; and Factor VIII may be provided to treat Hemophilia B. See, for example, Science 258 activated in the **method** of the invention, upon the application of ultrasound to the prodrug-containing microspheres with the...microspheres.

In addition, compounds which are generally thermally labile may be utilized to create toxic **free radical** compounds. Compounds with azolinkages, peroxides and disulfide linkages which decompose with high temperature are preferred...

...interaction of high energy sound with the gaseous precursor-filled microspheres to create cascades of **free radicals** from these prodrugs entrapped therein. A wide variety of drugs or chemicals may constitute these...

...hydrocarbon chain, where the double bond between the two nitrogen atoms may react to create **free radical** products in vivo.

Exemplary drugs or compounds which may be used to 35 create **free radical** products include azo containing compounds such as azobenzene, 2,21-azobisisobutyronitrile, azodicarbonamide, azolitmin, azomycin, azosemide...

...2,4-dimethylvaleronitrile).

A gaseous precursor-filled microsphere filled with oxygen gas should create extensive **free radicals** with cavitation. Also, metal ions from the transition series, especially manganese, iron and copper can...

...reactive oxygen intermediates from oxygen.

By encapsulating metal ions within the microspheres, the formation of **free radicals** in vivo can be increased. These metal ions may be incorporated into the microspheres as be incorporated into the gaseous precursor-filled microspheres to create **free radicals** on thermal stimulation.

By way of an example of the use of prodrugs, an acylated...The size of therapeutic containing liposomes can be adjusted, if desired, by a variety of **procedures** including extrusion, filtration, sonication, homogenization, employing a laminar stream of a core of liquid introduced...

...immiscible sheath of liquid, extrusion under pressure through 30 pores of defined size, and similar **methods** , in order to modulate resultant liposomal biodistribution and clearance.

The foregoing **techniques** , as well as others, are discussed, for example, in U.S. Patent No. 4,728...
...812, pp. 55-65 (1985); U.S. Patent No.

4,533,254; Mayhew et al., **Methods** in Enzymology, Vol. 149, pp. 64-77 (1987); Mayhew et al., Biochimica et Biophysica Acta...

...utilized. Filtration may either be utilized.

Filtration may be performed as part of the manufacturing **process** or during administration through an in-line filter.

The gaseous precursor-filled microspheres may be sized as a terminal step via a filtration **process** . A cascade filter comprising two or more serial filters, 10 micron followed by 8 micron...vivo. Indeed, knowing the expansion in microsphere diameter upon liquid to gaseous transition a filter **system** may be designed such that the particles or emulsion is sized via a **process** of injection/filtration. Upon transition from the liquid to 5 gaseous phases, the appropriate sized...

...of the desired diameter.
The gaseous precursor-filled microspheres may be sized by a simple **process** of extrusion through filters. The filter pore sizes control the size distribution of the resulting...

...more preferably the filter assembly may be incorporated into the syringe itself during use. This **process** may be applied using differently sized filters, such that differently sized microspheres result.

The size...

...To provide therapeutic delivery to organs such as the liver and to allow differentiation of **tumor** from normal

tissue, smaller microspheres, between about 30 nanometers and about 100 nanometers in mean...are administered individually, rather than, for example, embedded in a matrix.

Generally, the therapeutic delivery **systems** of the invention are administered in the form of an aqueous suspension such as inSuitable antioxidants include tocopherol, ascorbic acid and ascorbyl palmitate.
Methods of controlled delivery of therapeutic compounds to a region of a patient involve the steps...

...determine the presence of the microspheres in the region; and
(iii) rupturing the microspheres using **ultrasound** to release the **therapeutic** compound in the region.

Using the gaseous precursor-filled microspheres of the present invention, ultrasonic...

...the intended use. As one skilled in the art would recognize, administration of therapeutic delivery **systems** of the present invention may be carried out in various fashions, such as intravascularly, intralymphatically...

...dosage forms. one preferred route of administration is intravascularly. For intravascular use, the therapeutic delivery **system** is generally injected intravenously, but may be injected intraarterially as well. The microspheres of the...magnetic field is used to
create heating. This can be accomplished with an external magnetic **field** (i.e. the **magnet** outside the patient) and ferromagnetic probes implanted within the patient, e.g.

within a **tumor** . As a microspheres flow through the vessels within the **tumor** they will encounter heat in the region due 35 to the magnetic field oscillation. The...

...the mucosal surface of the colon) light energy can also be quite useful.

The preferred **method** of performing site directed drug delivery with the gaseous precursor microspheres is to apply energy...

...the body temperature (e.g. 37 C) tend to accumulate within diseased or ischemic tissue. **Tumors** are often ischemic, as 5 are infected areas of myocardium, brain, and other tissues.

the...the
presence of the microspheres in the region, and the microspheres are then ruptured using **ultrasound** to release the **therapeutics** in the region.

The patient may be any type of animal, but is preferably a...

...patient, or a particular area or portion of the

patient. For example, by using the **method** of the invention, therapeutic delivery may be effected in a patient's heart, 35 and a patient's vasculature (that is, venous or arterial **systems**). The invention is also particularly useful in delivering therapeutics to a patient's left heart...

...spleen and kidney regions of a patient, as well as other regions, using the present **methods** .

US005580575A

United States Patent [19]

Unger et al.

[11] Patent Number: 5,580,575

[45] Date of Patent: Dec. 3, 1996

[54] THERAPEUTIC DRUG DELIVERY SYSTEMS

[75] Inventors: Evan C. Unger; Thomas A. Fritz; Terry Matsunaga; VaradaRajan Ramaswami; David Yellowhair; Guanli Wu, all of Tucson, Ariz.

[73] Assignee: ImaRx Pharmaceutical Corp., Tucson, Ariz.

[21] Appl. No.: 76,250

[22] Filed: Jun. 11, 1993

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 716,899, Jun. 18, 1991, abandoned, and a continuation-in-part of Ser. No. 717,084, Jun. 18, 1991, Pat. No. 5,228,446, which is a continuation-in-part of Ser. No. 569,828, Aug. 20, 1990, Pat. No. 5,088,499, which is a continuation-in-part of Ser. No. 455,707, Dec. 22, 1989, abandoned.

[51] Int. Cl.[6] A61K 9/127

[52] U.S. Cl. 424/450

[58] Field of Search 424/450; 425/402.2; 436/829

[56] References Cited

U.S. PATENT DOCUMENTS

3,532,500	10/1970	Priest et al.	96/91
3,873,564	3/1975	Schneider et al.	260/309.6

(List continued on next page.)

FOREIGN PATENT DOCUMENTS

0107559	5/1984	European Pat. Off. .
0231091	1/1987	European Pat. Off. .
0272091	6/1988	European Pat. Off. .
0324938	6/1988	European Pat. Off. .
0338971	10/1989	European Pat. Off. .
0361894	4/1990	European Pat. Off. .
0216730	1/1991	European Pat. Off. .
0458745A1	11/1991	European Pat. Off. .
0314764B1	9/1992	European Pat. Off. .
0554213A1	8/1993	European Pat. Off. .
63-60943	3/1988	Japan .
2193095	3/1988	United Kingdom .
80/02365	11/1980	WIPO .
82/01642	5/1982	WIPO .
US85/01161	3/1985	WIPO .
86/00238	1/1986	WIPO .
86/01103	2/1986	WIPO .
89/05040	6/1989	WIPO .
90/04943	5/1990	WIPO .
91/00086	1/1991	WIPO .
91/15244	10/1991	WIPO .
92/10166	6/1992	WIPO .
92/17212	10/1992	WIPO .
93/05819	1/1993	WIPO .
93/20802	3/1993	WIPO .
93/06869	4/1993	WIPO .
93/13809	7/1993	WIPO .

OTHER PUBLICATIONS

Kost et al. Ultrasonic Modulated Drug Delivery Systems *Polymers in Medicine II* Plenum Press New York 387–396.

Brown et al. Transdermal Delivery of Drugs *Ann. Rev. Med.* 1988 39:221–229.

Santaella et al. Extended in vivo blood circulation time of fluorinated liposomes *FEBS* 1993 336:481–484.

Gramiak et al., *Radiology,* "Detection of Intracardiac Blood Flow by Pulsed Echo–Ranging", pp. 415–418 (1971).

Feigenbaum et al., *Circulation,* "Identification of Ultrasound Echoes from the left Ventricle by Use of Intracardiac Injections of Indocyanine Green", vol. XL1, pp. 615–621 (1970).

Stelmashok et al., *Koordinatsionnaya Khimiya,* vol.3, No. 4, pp. 524–527 (1977) (Russian language version).

(List continued on next page.)

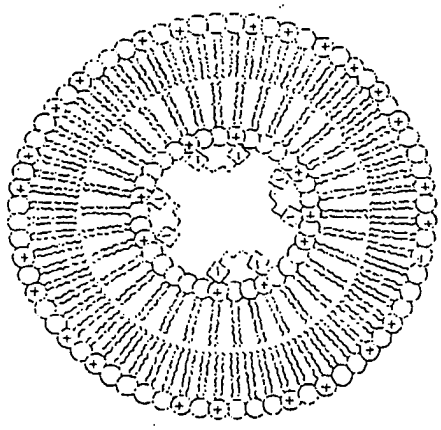
Primary Examiner—Gollamudi S. Kishore

Attorney, Agent, or Firm—Woodcock Washburn Kurtz Mackiewicz & Norris

[57] ABSTRACT

Therapeutic drug delivery systems comprising gas-filled microspheres comprising a therapeutic are described. Methods for employing such microspheres in therapeutic drug delivery applications are also provided. Drug delivery systems comprising gas-filled liposomes having encapsulated therein a drug are preferred. Methods of and apparatus for preparing such liposomes and methods for employing such liposomes in drug delivery applications are also disclosed.

17 Claims, 21 Drawing Sheets



25/3,K/65 (Item 65 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00435191 **Image available**
METHOD AND APPARATUS FOR ADMINISTRATION OF SUBSTANCES BY ULTRASOUND
PROCEDE ET APPAREIL PERMETTANT D'ADMINISTRER DES SUBSTANCES PAR ULTRA-SONS
Patent Applicant/Assignee:
TECHNION RESEARCH AND DEVELOPMENT FOUNDATION LTD,
IGER Yoni,
KIMMEL Eitan,
Inventor(s):
IGER Yoni,
KIMMEL Eitan,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9825655 A2 19980618
Application: WO 97IL405 19971212 (PCT/WO IL9700405)
Priority Application: IL 119827 19961213
Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 6497

Fulltext Availability:
Detailed Description

Detailed Description
... techniques are.

building up concentration gradients of the compounds to be administered; iontophoresis utilizing an **electromagnetic field** carried out both to increase the driving force of the administered substance and to cause...

...of internal organs, sterilization, degassation, superficial eye-lens-epithelium surgery, bile-stone perforation and anti- **cancer** treatment.

Ultrasound is also used for facilitation of transport of various compounds across tissues, typically...been reported probably since it results in irreversible damage to the tissue and in massive **cell - death** . Similarly, irreversible damage occurs in non-biological membranes of e.g., polyethylene or elastomer (for...be considered as not constituting an irreversible damage is an effect which should never exceed **necrosis** followed by loss of the superficial pavement cells at the irradiated zone, i.e. a...irradiation-activated compounds are several substances known to be activated by irradiation and to release **free radicals** . They are therefore used in medicine to cause damage to the surrounding tissues. Several substances are activated by light, in photodynamic therapy, in order to selectively destroy target tissue, typically **neoplasmic** tissue (Orenstein et aL, Br. J **Cancer** , 73:937-944, (1996)). There have been reports (Miyoshi et aL, Radiat. Res., 143:194-202 (1995)) of **cancer** treatment based on the combined effect of a photosensitizer and ultrasound which apparently is capable...appeared normal. Application of 1.5 W/CM2 for 50 see. (Fig. 2) resulted in **necrotic** superficial cells. Remnants

of outer cells are visible while cells
positioned at a deeper level...mucus,
surrounding the epithelium. Formation of holes in cell membranes and
membranes rupturing, up to **necrosis** , of the 2-3 outermost layers of the
epidermis (the outermost 20-30
performed for...

25/3,K/57 (Item 57 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00503479
ENHANCED TRANSPORT USING MEMBRANE DISRUPTIVE AGENTS
AMELIORATION DU TRANSPORT PAR L'UTILISATION D'AGENTS DE RUPTURE DE MEMBRANES
Patent Applicant/Assignee:
UNIVERSITY OF WASHINGTON,
UNIVERSITY OF MASSACHUSETTS,
Inventor(s):
HOFFMAN Allan S,
STAYTON Patrick,
PRESS Oliver,
TIRRELL David,
MURTHY Niren,
LACKEY Chantal,
CRUM Lawrence A,
MOURAD Pierre D,
PORTER Tyrone M,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9934831 A1 19990715
Application: WO 99US122 19990105 (PCT/WO US9900122)
Priority Application: US 9870411 19980105
Designated States: AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 13074

(US) 2001/0007666

Fulltext Availability:
Detailed Description

Detailed Description
... treat 30 genetic disorders, cause mutations in the genetic material in various cells, such as **tumor** cells, and bind to or interact with various sites in the cells to cause an...

...specificity, immunotoxins have been prepared that include the toxin conjugated to an antibody that targets **tumor** -associated antigens. Immunotoxins have had limited success as therapeutics, however, in part due to the inadequacy of penetration into **tumor** nodules and ineffective delivery of the toxin into cytosolic ribosomes.

It is often difficult to...until it is trafficked to a lysosome for degradation.
5 Press, O. W. et al., **Cancer** Research, 48: 2249-2257 (1988). Endosomes are
membrane bound phospholipid vesicles which function in intracellular...
enhancing transdermal or transmucosalpermeation
in the presence of physical stimuli such as ultrasonic, electric or **electromagnetic fields** .

Peptides
Peptides which lose their charge at a lower pH and become hydrophobic, thereby altering...polyacrylic acid graft copolymers can be prepared, for example, by polymerizing an Nacryloxysuccinimide monomer via **free radical** polymerization, reacting the resulting poly-(N-hydroxysuccinimide) (poly-NHS) with a

desired mole ratio
of...treatment which enhances the efficacy of the treatment, such as application of ultrasound, an electrical **field** , a **electromagnetic field** ,
iontopheresis, electroporation or a combination thereof
Ultrasound
Ultrasound will typically be applied using devices which...

...of
membrane disrupting agent can be determined empiracally, measuring cavitation (acoustically or by production of **free radicals** or chemical tracers such as iodine) or by measuring transport or release of material from...oxygen
which is normally in the triplet state to singlet oxygen, which is a potent **cell killer** . The latter is particularly effective in transport of cytotherapeutic drugs into **tumor** cells.

II. Diagnostic and Therapeutic Agents
Any therapeutic agent, prophylactic agent or diagnostic agent can...

...genes encoding defective or missing proteins, or genes encoding a lethal protein.

Preferred compounds for **killing cells** include glycoprotein-based toxins such as ricin, the B chain of the diptheria toxin, and **cells** to be **killed** -. Since these toxins bind to virtually every cell via the B-chain, they lack the...

...delivered is a toxin, and the endocytosis
enhancing agent is an antibody targeted to the **cells** to be **killed** , the resulting
5 conjugate is an immunotoxin which can be effectively delivered to the cytosol...

...in the examples. When the RTA was added
by itself to the cell culture, no **cell death** was noted, presumably due to the
intracellular trafficking of the toxin to the lysosomes. When...

...of the mixture (at a fixed ratio of 3/1 PPAA/RTA)Iead to increasing **cell deaths** . The polymer by itself was not toxic to cells. These observations indicate that the polymer...individual, or tissue of origin), viral antigens (in the case of virally infected
cells), and **tumor** antigens. These molecules can be targeted using antibodies, preferably monoclonal antibodies, most preferably human monoclonal antibodies or humanized antibodies, or using receptor-specific ligands. **Tumor** antigens are useful as targets for antibody-conjugated chemotherapeutic or cytotoxic agents. These are not specific markers for **tumor** cells in most cases; rather, they are overexpressed on **tumor** cells
compared with normal tissue, or they are found in association with normal fetal tissue [CEA (Gold, et al., J. Exp. Med. 122, 467-481 (1965)), AFP (Abelev, Ady. **Cancer** Res. 14, 295-350 (1971)) or with normal progenitor
1 5 cells of that organ in the adult (CEA). **Tumor** antigens can be localized in
the **tumor** interstitium, on the **tumor** cell membrane, or in the **tumor** cell
cytoplasm or nucleus.

Antigens that are found on cells in circulation and antigens

expressed on **tumor** neovasculature are readily accessible to intravenous (i.v.) administered reagents. Antigens that are expressed on the surface of tissue or **tumor** cells are readily accessible to intralesional (i.l.) or intraperitoneal (i.p.) administered conjugates. Antigens secreted into the **tumor** interstitium are most accessible to i.l. administration.

The membrane disruption agents can be conjugated by an alteration in cell activity - for example, by measuring **cell death** , by detection of a diagnostic agent, or by measuring transport of a particular analyte. The ...polymer-protein complexes. The percentage of hemolysis was pH and polymer concentration dependent.

Example 4: **Cell death** is enhanced when PPAA is mixed with a toxin.

Figure 5 is a schematic of...3 is when the endosomal pH of 5-6 triggers membrane lysis
andstep4iswhentheimmunotoxinisreleasedintothecytoplasm,leading
to **cell death** .

Objective.

Determine whether mixing PPAAc wtih ricin A chain (RTA) will
32
SUBSTITUTE SHEET (RULE...

...at a ratio of PPAAc:RTA = 3: 1.

Results.

As shown by Figure 6, no **cell death** was observed when the RTA
5 was added by itself to the cell culture, presumably...

...mixture (at a fixed ratio of
3:1 PPAA:RTA) led to increasing numbers of **cell deaths** . The polymer
by
itself was not toxic to cells. These results demonstrate that a mixture
...
...In the past decade, localized drug treatment and gene therapy in vivo
for disease and **cancer** has become a major area of research. One of the
major obstacles for this technique...

US 20010007666A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2001/0007666 A1

HOFFMAN et al. (43) Pub. Date: Jul. 12, 2001

(54) ENHANCED TRANSPORT USING MEMBRANE DISRUPTIVE AGENTS

(76) Inventors: ALLAN S. HOFFMAN, SEATTLE, WA (US); PATRICK STAYTON, SEATTLE, WA (US); OLIVER W. PRESS, SEATTLE, WA (US); DAVID TIRRELL, PASADENA, CA (US); NIREN MURTHY, SEATTLE, WA (US); CHANTAL LACKEY, SEATTLE, WA (US); LAWRENCE A. CRUM, ISSAQUAH, WA (US); PIERRE D. MOURAD, SEATTLE, WA (US); TYRONE M. PORTER, SEATTLE, WA (US)

Correspondence Address:
PATREA L. PABST
ARNALL GOLDEN & GREGORY
2800 ONE ATLANTIC CENTER
1201 PEACHTREE STREET
ATLANTA, GA 303093450

(*) Notice: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

(21) Appl. No.: 09/226,044

(22) Filed: Jan. 5, 1999

Related U.S. Application Data

(63) Non-provisional of provisional application No. 60/070,411, filed on Jan. 5, 1998.

Publication Classification

(51) Int. Cl.[7] A61K 9/00

(52) U.S. Cl. 424/400

(57) ABSTRACT

Compositions and methods for transport or release of therapeutic and diagnostic agents or metabolites or other analytes from cells, compartments within cells, or through cell layers or barriers are described. The compositions include a membrane barrier transport enhancing agent and are usually administered in combination with an enhancer and/or exposure to stimuli to effect disruption or altered permeability, transport or release. In a preferred embodiment, the compositions include compounds which disrupt endosomal membranes in response to the low pH in the endosomes but which are relatively inactive toward cell membranes, coupled directly or indirectly to a therapeutic or diagnostic agent. Other disruptive agents can also be used, responsive to stimuli and/or enhancers other than pH, such as light, electrical stimuli, electromagnetic stimuli, ultrasound, temperature, or combinations thereof. The compounds can be coupled by ionic, covalent or H bonds to an agent to be delivered or to a ligand which forms a complex with the agent to be delivered. Agents to be delivered can be therapeutic and/or diagnostic agents. Treatments which enhance delivery such as ultrasound, iontopheresis, and/or electrophereis can also be used with the disrupting agents.

25/3,K/54 (Item 54 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00535636 **Image available**
APPLICATION OF LIGHT AT PLURAL TREATMENT SITES WITHIN A TUMOR TO INCREASE THE EFFICACY OF LIGHT THERAPY
APPLICATION DE LUMIERE SUR PLUSIEURS SITES DE TRAITEMENT A L'INTERIEUR D'UNE TUMEUR AFIN D'ACCROITRE L'EFFICACITE DE LA PHOTOTHERAPIE
Patent Applicant/Assignee:
LIGHT SCIENCES LIMITED PARTNERSHIP,
Inventor(s):
CHEN James C,
Patent and Priority Information (Country, Number, Date): = (US) 6416531
Patent: WO 9966988 A1 19991229
Application: WO 99US9582 19990430 (PCT/WO US9909582)
Priority Application: US 98103761 19980624
Designated States: AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 10264

APPLICATION OF LIGHT AT PLURAL TREATMENT SITES WITHIN A TUMOR TO INCREASE THE EFFICACY OF LIGHT THERAPY
Main International Patent Class: **A61N-005/06**
Fulltext Availability:
Detailed Description
Claims

English Abstract
...of time at a plurality of sites (52) distributed within the abnormal tissue of a **tumor** (22). A clinical study has shown that a substantially greater volume of abnormal tissue in a **tumor** is destroyed by the extended administration of light therapy from a plurality of probes (50a ...

...than would have been expected based upon the teaching of the prior art. In this **process** , a plurality of light emitting optical fibers or probes are deployed in a spaced apart array. The greater volume of **necrosis** (38) in the **tumor** (22) is achieved due to one or more concomitant effects, including the inflammation of damaged...

...and resultant immunological response of the patient's body; and the collapse of the vascular **system** that provides oxygenated blood to portions of the **tumor** outside the expected fluency zone.

French Abstract
...tissu anormal dans une tumeur qu'on n'aurait pu s'y attendre avec la **technique** utilisee jusqu'ici. Selon la **technique** de l'invention, une pluralite de fibres optiques ou sondes emettrices de lumiere sont deployees...

...et la reponse immunologique provoquee dans l'organisme du patient; ainsi que l'effondrement du **systeme** vasculaire qui amene le sang oxygene aux parties de la tumeur situees a l'exterieur...

Detailed Description
APPLICATION OF LIGHT AT PLURAL TREATMENT SITES WITHIN A **TUMOR** TO INCREASE THE EFFICACY OF LIGHT THERAPY
Field of the Invention

The present invention generally relates to the use of light therapy to destroy abnormal tissue in a **tumor** , and more specifically, to the use of multiple light sources disposed at spaced-apart treatment sites within a **tumor** to render the therapy.

Background of the Invention
Abnormal tissue in the body is known...

...perfused into a treatment site to a much greater extent than surrounding tissue. For example, **tumors** of the pancreas and colon may absorb two to three times the volume of these dyes, compared to normal tissue. Once pre-sensitized by dye tagging in this manner, the **cancerous** or abnormal tissue can be destroyed by irradiation with light of an appropriate wavelength or...

...an absorbing wavelength or waveband of the dye, with minimal damage to normal tissue. This **proce**dure , which is known as photodynamic therapy (PDT), has been clinically used to treat metastatic breast **cancer** , bladder **cancer** , lung **carcinomas** , esophageal **cancer** , basal cell **carcinoma** , **malignant** melanoma, ocular **tumors** , head and neck **cancers** , and other types of **malignant** **tumors** . Because PDT may selectively destroy abnormal tissue that has absorbed more of the dye than normal tissue, it can successfully be used to kill the **malignant** tissue of a **tumor** with less effect on surrounding benign tissue than alternative treatment **procedures** .

The effectiveness of PDT for treating **tumors** has become increasingly more evident to the medical community. Each year, numerous papers are published...

...out to explore how PDT can more effectively be used and to better understand the **processes** by which PDT destroys abnormal cells. Much of the prior art discloses the use of...

...a patient or to an internal site within the patient's body. Penetration of a **tumor** by the optical fiber is achieved either through a small incision in the overlying dermal layer, or directly, if the **tumor** is surgically exposed.
Most applications of PDT are conducted using a single optical fiber to...

...the fiber.

Light emitted through the diffuser more fully illuminates a treatment site within a **tumor** in which the optical fiber has been inserted.

Research has been conducted to measure the...of tissue (as reported in "In Vivo Measurement of the Optical Interaction Coefficients of Human **Tumors** at 630 nrn," 1. Driver, C.P.

Lowdell, and D.V. Ash, Phys. Med. Biol...

...will be effective in destroying abnormal tissue in only a relatively small volume within a **tumor** . To treat larger **tumors** , multiple light treatment sites would be expected to linearly expand the volume as a function...

...light treatment sites used, i.e., the total volume of the effective zone in a **tumor** treated with the multiple optical 3.5 fibers should be equal to the product of...
...the number of sites. In a paper entitled "Photodosimetry of Interstitial

Light Delivery
to Solid **Tumors** ," M.C. Fenning, D.Q. Brown, and J.D. Chapman, Medical Physics, Vol. 21, No...

...a 2cm laterally diffusing optical fiber placed within a plastic brachytherapy needle implanted into a **tumor** . The radial falloff of intensity with distance from single
fibers was used to determine light...

...perpendicular to the single-fiber and various multiple-fiber configurations.

Relative light intensities measured along **tumor** tracks were compared with those predicted by the 2D photodosimetry evaluation and were found to...

...optical fiber spacings of at least one cm produced relatively uniform light fields (+/- 20%) in **tumor** planes perpendicular to the optical fibers. At line 32 ...second column on page 1 155 of the paper, it is noted that.
For human **tumors** with light attenuating properties similar to the R3327-H **tumor** , the heterogeneity of light dose in **tumor** volumes delivered by a multifiber illuminator with 1 cm spacings will be considerably greater than +/- 20%. Illumination of **tumors** by such **procedures** will produce relatively large variations in biological effect by interstitial PDT. Furthermore, to expose all **tumor** tissue to a minimum light dose required for a specific biological effect, large fractions of the **tumor** would of necessity be overdosed.

While this may not seriously impact upon **tumor** response, it will limit the volume of solid **tumor** which can be treated with a specific time by a specific light source. Laser output...

...illumination of superficial lesions
in clinical studies to date. Nevertheless, to successfully scale up this **procedure** for the treatment of bulky human **tumors** , laser output intensity and **tumor** volume will determine the time required to deliver a curative light dose.

The paper further concludes that more than seven optical fibers may be required to properly treat a **tumor** with PDT, to guarantee that adequate light is delivered, particularly to the periphery of a **tumor** , due to the rapid falloff of light at the edge of the illuminated field. The reference thus teaches or suggests that the effect of PDT on a human **tumor** , particularly one of larger size, will be limited to the region of the **tumor** directly viably illuminated by the plurality of optical fibers and implies that it will be necessary to repeat the treatment to different areas of the **tumor** by moving the plurality of the optical fibers so that direct illumination of a greater...

...cells relies upon the conversion of molecular oxygen to singlet oxygen and the release of **free radicals** by the light activated dye. In "How Does
Photodynamic Therapy Work?" by B.W. Henderson...are vasoactive, either constrictive or dilatory, and it is believed that they induce vascular damage.

Tumor necrosis factor (TNF) is also released, and it too can cause vascular damage. The degree of...

...the level of the circulating photoreactive agent. This reference reports that
vascular damage in a **tumor** microenvironment induces hypoxic **tumor** cell
fractions. A key conclusion stated in the paper is that the "rapid shift of...

...they are protected from further PDT damage due to the oxygen limitation of the photodynarnic **processes** , is potentially limiting to direct **tumor** cell photodestruction." In essence, this statement indicates that efficacy of PDT in destroying **tumor** cells quickly diminishes after the light activation due to the self limiting effects of hypoxia...

...flow to the treatment site, which is necessary to supply additional molecular oxygen to the **tumor** cells for use in generating more singlet oxygen.

Also reported in the last cited reference...

...days following the therapy, concurrent with severe inflammatory symptoms. The relative extent of abnormal cell **necrosis** caused by generation of singlet oxygen and **free** **radicals** compared to that resulting from the 1 5 immune response is not clear from the...

...treatment sites within a patient's body.
Such probes can be implanted interstitially within a **tumor** to administer PDT for many hours or days. As necessary, repetitive infusions of a suitable photoreactive agent can be made to sensitize the abnormal cells comprising the **tumor** so that they are susceptible to being destroyed by the ...arises in regard to the efficacy of such an approach to treating a relatively large **tumor** .

In view of the teaching of the art discussed above, one would be led to ...

...low intensity light sources on an interstitial probe would lack adequate penetration into a large **tumor** mass to treat more than a relatively small portion of the **tumor** - even if plural probes of this type were used. In addition, the prior art suggests that extended PDT delivered to a treatment site will not be effective in a
large **tumor** due to the hypoxia resulting from vascular damage and the vasculature constriction that occurs soon after the PDT commences.

315 Application of PDT to a larger **tumor** would seem to require that a plurality of optical fibers spaced sufficiently close together and of sufficient number be inserted into the **tumor** to ensure that the light intensity between the optical fibers is substantially uniform throughout the volume of the **tumor** being treated. However, in view of the teaching of the prior art, implanting sufficient numbers...

...such uniform illumination does not seem to be a practical approach for treating a larger **tumor** .

The expected effective zone of PDT would seem to be too limited due to the...

...of light into the tissue to justify the use of PDT to treat a large **tumor** .

Summary of the Invention

Contrary to the suggestion of the prior art, it appears that PDT can be successfully used for treating larger **tumor** masses, and that 'the depth of light penetration into **tumor** tissue when effecting PDT is not so limiting as indicated in the prior art, in...

...extent of the effectiveness of the therapy.

Indeed, the effective zone of PDT in large **tumors** has been found to be much larger than the volume of the **tumor** into which light administered has previously been found to penetrate. Furthermore, the effectiveness of the PDT in treating a larger volume of a **tumor** appears to be more dependent upon a pattern in which light emitting sites are arrayed in the **tumor** than previously known.

In accord with the present invention, a **method** is defined for destroying
abnormal tissue in a **tumor** within a patient's body using an extended light therapy and at least one concomitant effect thereof. The **method** includes the step of administering a photoreactive agent to the abnormal tissue. The photoreactive agent...

...the absorption waveband of the
photoreactive agent is administered to a treatment zone in the **tumor** . A pattern in which the light is administered to the turnor defines the treatment zone, and this zone preferably encompasses a substantial portion of the **tumor** not penetrated by the light being administered. The **method** provides for continuing ...thereby. Furthermore, the extended period of light therapy indirectly destroys the substantial portion of the **tumor** that is not penetrated by the light being administered by inducing at least one concomitant 3.5 effect that destroys the abnormal tissue comprising the substantial portion of the **tumor** .

In one case, the concomitant effect arises because the destruction of the abnormal tissue in the treatment zone deprives the substantial portion of the **tumor** from receiving oxygen. The abnormal tissue in the substantial portion of the **tumor** is thus destroyed due to oxygen depletion.

In another instance, the concomitant effect arises because...

...that is activated by the light being administered diffuses into the substantial portion of the **tumor** that is not penetrated by the light. This photoreactive agent that is thus activated then destroys the abnormal tissue in the substantial portion of the **tumor** not directly penetrated by the light.

In yet another instance, the concomitant effect arises becau se the light therapy causes **necrosis** of the abnormal tissue in the treatment zone, which causes either an immune response or...

...the patient's body that destroys the abnormal tissue in the substantial portion of the **tumor** not directly penetrated by the light.
In still another instance, the concomitant effect arises because...

...vascular collapse, stasis, or occlusion, so that blood flow to the substantial portion of the **tumor** that is not directly penetrated by the light is terminated, causing the abnormal tissue in that substantial portion to die.

In one embodiment of the **method** , the light is administered through an

optical fiber from a source that is external to the patient's body. The **method** further preferably includes the step of implanting a plurality of probes for administering the light into the **tumor** at spaced-apart locations within the treatment zone. In one embodiment, the light is then ...

...not more than about 3 cm from each of the plurality of probes.

In the **method** , the light administered to the treatment zone produces singlet oxygen, which depletes oxygen from the substantial portion of the **tumor** that is outside the treatment zone, causing a gradient of hypoxia and anoxia in that portion of the **tumor** , which leads to a destruction of the abnormal tissue contained therein.
The **method** may include further steps. Specifically, in one embodiment, the light is emitted into the **tumor** in a first direction from each of the plurality of probes, relative to the probe from which the light is emitted. Next, the **method** provides for terminating emission of light into the **tumor** in the first direction and emitting light into the **tumor** in a second direction from each of the plurality of probes. The second direction is...

...probes. Preferably, in one embodiment, the first direction is directed toward a perimeter of the **tumor** , and the second direction is directed toward an interior of the **tumor** . By first destroying the perimeter of the **tumor** , the interior portion of the **tumor** is more readily destroyed due to the one or more concomitant effects.

Brief Description of...

...first embodiment of the present invention for administering light to a treatment
site within a **tumor** in a patient's body;
FIGURE 2 is a plan view of the **tumor** shown in FIGURE 1, illustrating the positions of probes and the radial depth to which light emitted thereby directly
penetrates into the **tumor** ;
FIGURE 3 is a side elevational view of a second embodiment of the present invention, showing the **tumor** with a plurality of light emitting implanted
probes inserted therein;
FIGURE 4 is a plan view of the **tumor** shown in FIGURE 3, illustrating the direct light penetration pattern for each of the probes...

...FIGURE 7 is a schematic diagram comparing a depth of direct light penetration in a **tumor** to a depth of **tumor** **necrosis** caused by one or more
secondary effects;
FIGURE 8 is a plan view of a **tumor** showing a plurality of probes that selectively emit light in one of two different directions...

...is selectively energized;
FIGURE 1 IA is a schematic side elevational view of a retroperitoneal **tumor** within a patient's body that was treated in accord with the present
invention;
FIGURE 1 1 B is a plan view of the **tumor** of FIGURE I IA showing the disposition of a plurality of probes used to administer PDT to the **tumor** , the expected fluence zone, and the substantially greater expanded **necrotic** zone
actually achieved;

FIGURE 12 is a side elevational view of ...of light emitting probes for enhancing the effect of PDT in treating a large volume **tumor** ; and FIGURE 13 is a side elevational view of a second pattern of light emitting probes for enhancing the effect of PDT in treating the large volume **tumor** .

Description of the Preferred Embodiment
With reference to FIGURE 1, the present invention is illustrated in connection with treating a **tumor** 22 that is disposed within a patient's body 20.

Tumor 22 is relatively large, having a length of approximately 7 to 10 cm and a transverse width of about 7 cm in this exemplary illustration. The **tumor** is disposed below a dermal layer 24, for example, within the patient's abdominal cavity.

In the present invention, PDT plays an important role is destroying abnormal tissue comprising **tumor** 22. As is done when rendering conventional PDT, a photoreactive agent is administered to the...
...by in.ection and is selectivel preferentially absorbed by the abnormal tissue of
i y
tumor 22. Thereafter, using a surgical **procedure** to access **tumor** 22 through dermal layer 24, or using an endoscopic **procedure** with minimally invasive impact, a plurality of optical fibers 30a-30e are inserted into the interior of **tumor** 22 in a spaced-apart array so that the optical fibers are arranged in a pattern that is more likely to increase the effectiveness of the therapy administered to the **tumor** . A laser light source 26 produces light lying within the light absorption waveband of the...

...optical fibers to their distal ends, which have been inserted interstitially into the interior of **tumor** 22.

In the embodiment illustrated in FIGURE 1, cladding 32 is removed from approximately the...

...through the sides and through the distal ends of the optical fibers inserted into the **tumor** . Light emitted by the exposed distal ends of each of these optical fibers penetrates **tumor** 22 to an effective depth of less than 1.5 cm. The penetration depth of the emitted light into the **tumor** determines a generally cylindrical expected fluence zone ... exposed portions of cores 34 from
which the cladding has been removed are inserted into **tumor** 22, generally forming a circle in which the expected fluence zones 36 around each optical...

...each of the optical fibers and partly by the nature of the abnormal tissue in **tumor** 22. Measurements in the prior art indicate that for most **tumor** tissue, the maximum effective depth of light penetration (at a wavelength of 600 - 700 nm) within **tumor** tissue is less than 1.5 cm. Furthermore, the effective depth of the expected fluence...

...conventional PDT, light at relatively high intensity is delivered to a treatment site within a **tumor** through one or more optical fibers for a relatively short period of time, typically much...

...to be limited to the expected fluence zones, i.e., to the volume of the **tumor** directly illuminated by the light emitted from the optical fiber(s). In contrast, in the...

...additional photoreactive agent may be administered to the patient, depending upon the size of the **tumor** , the type of photereactive agent used, and other conditions unique to each patient.

By administering...

...using the plurality of optical fibers shown in FIGURES I and 2, a substantially expanded **necrotic** zone 38 in **tumor** 22 should be achieved in which the abnormal tissue well outside the expected fluence zones of each of the optical fibers is destroyed.

The substantially greater volume of **necrotic** zone 38 in **tumor** 22 is believed to be due to one or more causes that ...zones around each optical fiber is destroyed due to one or more other factors or **processes** that differ from the **process** involved in conventional short-term PDT.

The extended duration PDT of the present invention is...

...extended period of light therapy is not triggered by the presence of abnormal tissue in **tumor** 22, it is believed that the extended period of light therapy administered under the present...

...tissue outside the expected fluence zones is attacked and destroyed by the patient's own **system** .
Another possible cause for the expanded volume in which **necrosis** of the abnormal tissue in **tumor** 22 will occur in connection with the present invention is the oxygen depletion outside the...

...zones that arises due to the generation of singlet oxygen as light is administered to **tumor** 22 through the optical fibers. Since the light is administered for an extended period of...

...expected fluence zones, causing a gradient of hypoxia and anoxia in the portion of the **tumor** that is not directly illuminated by light. This continuing photodynamic
transformation of molecular oxygen to...

...produced in the expected fluence zones diffuses or circulates into the larger volume of the **tumor** outside these zones.

Another possible cause of the expanded **necrotic** zone achieved by the present invention is the spread of activated photoreactive agent from within the expected fluence zones into other portions of the **tumor** outside these zones. Once activated by light administered during the extended period of light therapy...
...agent diffuses and circulates outside the expected fluence zones and into other portions of the **tumor** , where it may destroy abnormal tissue. It is believed that the photoreactive agent that has...prior art has taught should not be possible.

A further possible cause of the expanded **necrotic** zone obtained with the
present invention is a vascular stasis, collapse, or occlusion occurring outside...

...has not previously been observed or disclosed as giving rise to an expanded volume of **necrosis** in a **tumor** following an extended period of light therapy.

There is another possible explanation for the much...

...destroyed in accord with the present invention. Light applied to the abnormal tissue in the **tumor** is likely to be scattered along random paths within the tissue that may penetrate to...

...to have much effect when administered for the relatively short duration of a conventional PDT **procedure** , but may have a much more pronounced effect when delivered for the extended duration of ...

...conversion of molecular oxygen to singlet oxygen occurs at a much greater depth within the **tumor** than the expected fluence zone would indicate.

The present invention is clearly not limited to...

...light source, an arc lamp, or other source of light that is conveyed to a **tumor** through an optical fiber (or light pipe), or is disposed on a probe that is inserted into the **tumor** . Since the light is administered to the **tumor** for an extended period of time, it is generally preferable to implant the source of the light directly into the **tumor** at a plurality of ...a plurality of LEDs or other light sources disposed therein are preferably implanted within the **tumor** and energized using an implanted power source. An implanted conductive coil can be energized by...

...an external coil connected to an alternating current source, or by otherwise producing a varying **electromagnetic field** outside the patient's body that is coupled to the implanted coil. U.S. Patent...
...herein by reference.

FIGURE 3 illustrates implanted probes 50a-50e, which have been inserted into **tumor** 22 in a generally circular pattern. As shown more clearly in FIGURE4, each of probes50a...

...or extended period of time that enables abnormal tissue in a substantially larger volume comprising **necrotic** zone 38 of **tumor** 22 to be destroyed. **Necrotic** zone 38 includes central zone 56, which is surrounded by expected fluence zones 52. Each...

...aspect of the present invention relates to the effect on central zone 56 of the **necrosis** of abnormal tissue occurring in expected fluence zones 52 due to PDT. Since these expected...be destroyed due to oxygen and nutrient starvation. However, since the present invention will produce **necrotic** zone 38 that extends radially outward of the expected fluence zones, the present invention is...

...an expected fluence zone I 10 extending approximately 1.5 centimeters, and a depth of **tumor** **necrosis** 112 that is greater than five centimeters in accord with the present invention. Optical fibers 98, light can be directed toward the interior of a **tumor** , and light directed toward the periphery of the **tumor** can be minimized, thereby 3.5 avoiding exposure of normal tissue outside the limits of the **tumor** to the light.

The directional emission of light from optical fibers 90 and 98 can...

...the extended period in accord with the present invention.

With reference to FIGURE 8, a **tumor** 120 is illustrated in a plan view; a
plurality of probes 122 that emit light of an appropriate waveband have been implanted in spaced-apart array within the **tumor** , generally defining a circle.

Each of probes 122 includes two separately energizable groups of light...

...light rays I26 are emitted that are generally directed toward the perimeter or periphery of **tumor** 120. As a further aspect of the present invention, it is contemplated that by initially administering light rays 126 directed towards the periphery of the **tumor** , destruction of the abnormal tissue comprising **tumor** 120 will occur first around the periphery of the **tumor** . Thereafter, light sources 124b are energized, and light sources 124a are de-energized. Light sources 124b emit light rays 128 that are directed toward the inner portion of **tumor** 120. **Necrosis** of the abnormal tissue around the periphery of the **tumor** should tend to cause vascular stasis, collapse, or occlusion of the vascular structure providing oxygenated blood to the inner portion of **tumor** 120.

Accordingly, the extended light therapy provided by light rays 128 should continue the destruction of abnormal tissue within the interior of **tumor** 120 and the actual **necrotic** zone will be extended as a result of one or more of the
concomitant factors discussed above. An enhanced **necrosis** volume within **tumor** 120 is thus achieved using this two pronged light therapy. As a further benefit, less...coil, electromagnetically coupled to an external source of power, as noted above.

FIGURE 10 illustrates **tumor** 120 in which probes 180 are inserted generally in a radial direction within the **tumor** . Probes 180 each include a first set of LEDs 186 that are disposed adjacent an...

...from each of probes 180 in the region radially closer to a perimeter 130 of **tumor** 120. After the photoreactive agent absorbed by **tumor** 120 has been activated to destroy abnormal tissue adjacent the periphery of the **tumor** , first set of LEDs 186 are de-energized, and second set of LEDs 188 are energized, emitting light rays 194, which are incident on the inner portion of **tumor** 120. The concomitant factors occurring as a result of the extended duration of light therapy provided by light rays 192 and 194 thereby destroy substantially all of **tumor** 120, even though the total volume of **tumor** 120 is substantially greater than the expected fluence zone for the light sources on probes...

...connection with an actual in vivo clinical test on a patient to treat a retroperitoneal **tumor** 200, generally
shaped as shown in FIGURES II A and II B. **Tumor** 200 had been treated previously with chemotherapy and with **radiation therapy** . Also, attempts had been made to surgically remove it, but **tumor** 200 had been resistant to each of these conventional forms of treatment. In fact, at the time the clinical study was undertaken, **tumor** 200 had grown through a dermal layer 202 so that ...probes 206 and two probes 208 were initially inserted into protruding portion 204 of the **tumor** .

Since this portion of the **tumor** was fully exposed, it was not necessary to surgically or endoscopically implant probes 206 and...
...hours prior to energizing probes 206 and 208. Probes 208 were inadvertently pulled from the **tumor** and were de-activated after approximately 18 hours, leaving the remaining four probes 206 in...

...total of 48 hours.

Four weeks following the administration of the extended light therapy to **tumor** 200, **necrosis** in the protruding portion was observed up to approximately five centimeters away from the point where the nearest probe had been disposed.

The maximum depth of the **necrosis** within protruding portion 204 was 5 cm
beyond the distal tip of any of the probes. Thus, the extent of the **necrosis** observed in **tumor** 200 was substantially and unexpectedly greater than would have been expected based upon the teachings of the prior art. This extensive
volume of **necrosis** is believed to have been caused by one or more of the
concomitant factors discussed above. The substantially greater volume of **necrosis** , extending both radially and in depth well beyond the expected fluence zones of the probes...

...zones.

FIGURES 12 and 13 illustrate two further exemplary configurations for placing probes within a **tumor** 220. In these exemplary configurations, the abnormal tissue comprising the **tumor** is treated with an appropriate photoreactive agent prior to administration of the extended light therapy ...

...inwardly extending distal ends.

A probe 222d is inserted generally within the central region of **tumor** 220, adjacent its upper surface. Leads 224 extend from the probes to a remote power source (not shown) for energizing the probes so that they emit light into the **tumor** .

By positioning probe 222d transversely within the central portion of the **tumor** , the light pattern provided by the probes should enhance the volume of **necrosis** resulting from one or more of the concomitant factors discussed above. The interior of **tumor** 220 should be deprived of oxygen due to the destruction of the vascular **system** surrounding it and this factor should also enhance the destruction of abnormal tissue resulting from the exemplary configuration of
probes 226a-226e within **tumor** 220 shown in FIGURE 13. Again, leads 224 couple these probes to a remote power source (not shown). In this embodiment, each of the probes are generally inserted into **tumor** 220 so that their longitudinal axes are generally parallel with each other. Probe 226c is inserted into the center of **tumor** 220 and includes light sources adjacent its proximal -and distal ends that are energized to...

...of the surrounding abnormal tissue. The oxygen and
nutrient supply to the internal portion of **tumor** 220 is thus cut off due to the **necrosis** of the vascular **system** around the periphery of the **tumor** .

It will be apparent that the probes and leads in the above examples may be...

Claim

I . A **method** for destroying abnormal tissue in a **tumor** within a patient's body using an extended light therapy and at least one concomitant...

...and having a characteristic absorption waveband;
(b) administering light to a treatment zone in the **tumor** that is determined by the penetration depth of the light into the **tumor** along a direct path, said light having a waveband corresponding to the absorption waveband of the photoreactive agent, a pattern in which said light is administered to the **tumor** defining a shape of the treatment zone so that the treatment zone encompasses a substantial portion of the **tumor** not penetrated by the light being administered along a direct path and is thus outside...

...absorbed thereby, said extended period of light therapy indirectly destroying said substantial portion of the **tumor** that is not penetrated by the light being administered by inducing said at least one concomitant effect that destroys the abnormal tissue comprising said substantial portion of the **tumor** .

2 The **method** of Claim 1, wherein destruction of the abnon-nal tissue in the treatment zone deprives said substantial portion of the **tumor** from receiving oxygen, said abnormal tissue in said substantial tissue thus being destroyed due to oxygen depletion.

3 The **method** of Claim 1, wherein the photoreactive agent in the treatment zone that is activated by the light being administered thereto diffuses into said substantial portion of the **tumor** that is not penetrated by the light, said photoreactive agent that is thus activated being operative to destroy the abnormal tissue in the substantial portion of the **tumor** .

4 The **method** of Claim 1, wherein the light therapy causes **necrosis** of the abnormal tissue in the treatment zone, which causes one of an immune response...

...the patient's body that destroys the abnormal tissue in said substantial portion of the **tumor** .

5 The **method** of Claim 1, wherein destruction of abnormal tissue in the treatment zone causes one of vascular collapse, stasis, and occlusion, so that blood flow to said substantial portion of the **tumor** is interrupted, causing the abnormal tissue in said substantial portion to die.

6 The **method** of Claim 1, wherein the light is administered through an optical fiber from a source that is external to the patient's body.

7 The **method** of Claim 1, further comprising the step of implanting a plurality of probes for administering the light into the **tumor** at spaced-apart locations within the treatment zone to determine the shape of the treatment zone.

8 The **method** of Claim 7, wherein ...at least one light source included on each of the plurality of probes.

9 The **method** of Claim 7, wherein the light is delivered to the plurality of probes through a...

...fibers from a source that is external to th e patient's body.

10 The **method** of Claim 7, wherein the depth at which light from each of said plurality of probes penetrates the **tumor** in the treatment zone

is not more than about 3 cm.

11 The **method** of Claim 1, wherein the light administered to the treatment zone produces singlet oxygen, which depletes oxygen from said substantial portion of the **tumor** , causing a gradient of a hypoxia and an anoxia in said substantial portion of the **tumor** that leads to a destruction of the abnormal tissue in said substantial portion of the **tumor** .

. The **method** of Claim 7, further comprising the steps of:

(a) emitting light into the **tumor** in a first direction from each of the plurality of probes, said first direction being...

...is emitted and not necessarily identical for each probe;

(b) terminating light emission into the **tumor** in the first direction; and

(c) emitting light into the **tumor** in a second direction from each of the plurality of probes, said second direction being...

...first direction for each of the probes from which the light is emitted.

13 The **method** of Claim 12, wherein the first direction is toward a perimeter of the **tumor** , and the second direction is toward an interior of the **tumor** , light emitted in the first direction causing destruction of the abnormal tissue toward the perimeter of the **tumor** from each probe, which improves an efficacy with which the light emitted in the second direction destroys the abnormal tissue.

14 A **method** for destroying abnormal tissue in a **tumor** within a patient's body using an extended light therapy that induces **necrosis** of the abnormal tissue beyond a zone over which the light therapy is administered,

comprising the steps of:

(a) administering a photoreactive agent to the abnon-nal tissue of the **tumor** , said photoreactive agent being preferentially absorbed by the abnormal tissue rather than by normal tissue...

...administering light to the abnormal tissue at a plurality of spaced-apart sites within the **tumor** through a plurality of probes that are inserted within the **tumor** , light from said plurality of probes only penetrating the abnormal tissue within a treatment zone comprising a limited volume of the **tumor** , said

light being within the characteristic light absorption bandwidth of the photoreactive agent, said light activating the photoreactive agent to destroy the abnormal tissue illuminated by the light by a **process** of photodynamic therapy;

and

(c) continuing the administration of light through the plurality of probes inducing a secondary effect that causes **necrosis** of the abnormal tissue outside the treatment zone, in a region of the **tumor** that is not penetrated by the light traveling in a direct path.

. The **method** of Claim 14, wherein the probes are inserted into the **tumor** in a pattern that at least partially surrounds said region of the **tumor** that is not penetrated by the light traveling in a direct path, destruction of the...

...the photodynarnic therapy causing one of a vascular stasis, collapse, and occlusion that induces the **necrosis** of the abnormal tissue outside the treatment zone.

16 The **method** of Claim 14, further comprising the step of directing the light from the plurality of...

...predefined direction to limit administration of the photodynamic therapy to a defined portion of the **tumor** .

17 The **method** of Claim 16, wherein the step of directing comprises the step of providing a reflective...

...of the probe to limit administration of the light in the predefined direction.

18 The **method** of Claim 14, wherein destruction of the abnormal tissue in the treatment zone due to the photodynamic therapy induced **necrosis** of the abnormal tissue outside the treatment zone due to one of an immune response and an inflammation.

19 The **method** of Claim 14, wherein the **necrosis** of the abnormal tissue outside the treatment zone is induced as a result of an...

...formation of singlet oxygen in the treatment zone during the extended treatment period.

20 - The **method** of Claim 14, wherein the **necrosis** of the abnormal tissue outside the treatment zone occurs due to a diffusion and circulation...

...the light enabling it to destroy the abnormal tissue outside the treatment zone.

21 The **method** of Claim 14, wherein the plura!,ty of probes emit light that is produced by at least one light source on each of the plurality of probes.

22 The **method** of Claim 14, wherein the plurality of probes are each coupled to a source of...

...patient's body by a different one of a corresponding plurality of optical fibers.

. The **method** of Claim 14, wherein a plurality of spaced-apart light sources are included within each of the plurality of probes.

24 The **method** of Claim 23, where different ones of the plurality of light sources on at least...

...the plurality of light sources that emit light in a second predefined direction.

25 The **method** of Claim 14, wherein the plurality of probes are transcutaneously inserted into the **tumor** .

26 The **method** of Claim 25, further comprising the step of energizing the plurality of probes from an external source of power by transcutaneous transfer of energy.

27 The **method** of Claim 14, further comprising the step of alternating a direction in which the light is administered to the **tumor** from each probe between at least a first and a second direction during the extended treatment period, said first direction being substantially different than the second direction.

28 The **method** of Claim 27, wherein the light is only administered in one of the first and...

...invention in which an exclusive right is claimed is defined by the following:

1 A **method** for destroying abnormal tissue in a **tumor** within a patient's body using an extended light therapy and at least one concomitant...

...aving a c aracteristic sorption waveband;
(b) administering light to a treatment zone in the **tumor** to a depth that
is determined by the penetration depth of the light into the **tumor** along a direct path, said light having a waveband corresponding to the absorption waveband of the photoreactive agent, a pattern in which said light is administered to the **tumor** defining a shape of the treatment zone, the treatment zone encompassing a substantial portion of the **tumor** not penetrated by the light being administered
along a direct path; and
(c) continuing to...

...absorbed thereby, said extended period of light therapy indirectly destroying said substantial portion of the **tumor** that is not penetrated by the light being administered by inducing said at least one concomitant effect that destroys the abnormal tissue comprising said substantial portion of the **tumor** .

2 The **method** of Claim 1, wherein destruction of the abnormal tissue in the treatment zone deprives said substantial portion of the **tumor** from receiving oxygen, said abnormal tissue in said substantial tissue thus being destroyed due to oxygen depletion.

3 The **method** of Claim 1, wherein the photoreactive agent in the treatment zone that is activated by the light being administered thereto diffuses into said substantial portion of the **tumor** that is not penetrated by the light, said photoreactive agent that is thus activated being operative to destroy the abnormal tissue in the substantial portion of the **tumor** .
AMENDED SHIEET (ARTICLE 19)

US006416531B2

(12) **United States Patent**
Chen

(10) **Patent No.: US 6,416,531 B2**
(45) **Date of Patent: *Jul. 9, 2002**

(54) **APPLICATION OF LIGHT AT PLURAL TREATMENT SITES WITHIN A TUMOR TO INCREASE THE EFFICACY OF LIGHT THERAPY**

(75) Inventor: **James C. Chen**, Bellevue, WA (US)

(73) Assignee: **Light Sciences Corporation**, Issaquah, WA (US)

(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/103,761**

(22) Filed: **Jun. 24, 1998**

(51) **Int. Cl.**[7] **A61B 18/20**

(52) **U.S. Cl.** **607/89**; 606/10; 606/13; 604/20; 128/898

(58) **Field of Search** 606/2, 3–19; 128/898; 607/88–96, 98–101; 604/20

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,336,809 A		6/1982	Clark	
4,651,739 A	*	3/1987	Oseroff et al.	607/88
5,000,752 A	*	3/1991	Hoskin	606/9
5,445,608 A	*	8/1995	Chen et al.	604/19
5,514,669 A		5/1996	Selman	514/63
5,715,837 A		2/1998	Chen	128/899

OTHER PUBLICATIONS

"Optical Dosimetry for interstitial photodynamic therapy" by Arnfield et al; Med Phys vol. 16 No. 4; Jul.–Aug./1989; pp. 603–608.*

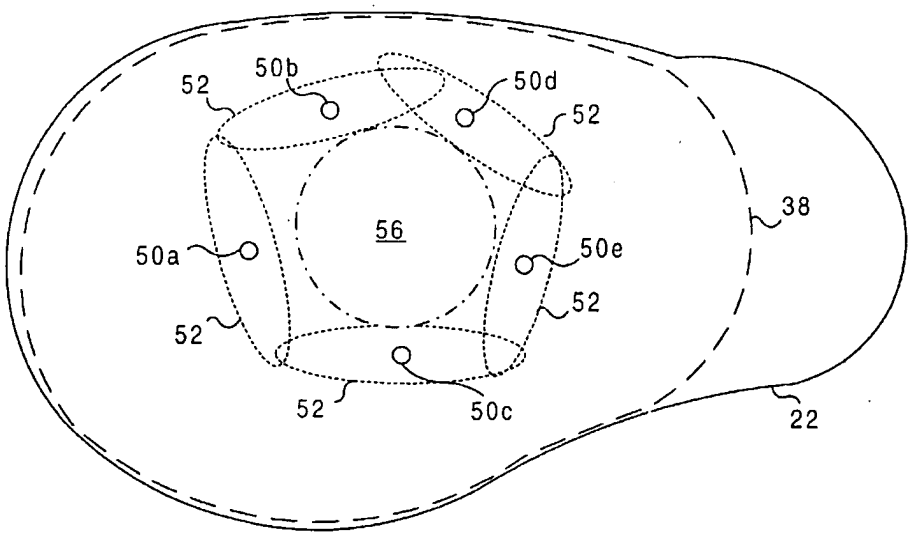
* cited by examiner

Primary Examiner—David M. Shay
(74) *Attorney, Agent, or Firm*—Ronald M. Anderson

(57) **ABSTRACT**

Light is administered during photodynamic therapy (PDT) for an extended period of time at a plurality of sites distributed within the abnormal tissue of a tumor. A clinical study has shown that a substantially greater volume of abnormal tissue in a tumor is destroyed by the extended administration of light therapy from a plurality of probes than would have been expected based upon the teaching of the prior art. In this process, a plurality of light emitting optical fibers or probes are deployed in a spaced-apart array. After a photoreactive agent is absorbed by the abnormal tissue, the light therapy is administered for at least three hours. The greater volume of necrosis in the tumor is achieved due to one or more concomitant effects, including: the inflammation of damaged abnormal tissue and resultant immunological response of the patient's body; the diffusion and circulation of activated photoreactive agent outside the expected fluence zone, which is believed to destroy the abnormal tissue; a retrograde thrombosis or vascular occlusion outside of the expected fluence zone; and, the collapse of the vascular system that provides oxygenated blood to portions of the tumor outside the expected fluence zone. In addition, is possible that molecular oxygen diffusing and circulating into the expected fluence zone is converted to singlet oxygen during the extended light therapy, causing a gradient of hypoxia and anoxia that destroys the abnormal tissue outside the expected fluence zone.

26 Claims, 7 Drawing Sheets



25/3,K/23 (Item 23 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00992681 **Image available**
THERMOTHERAPY VIA TARGETED DELIVERY OF NANOSCALE MAGNETIC PARTICLES
THERMOTHERAPIE EFFECTUEE AU MOYEN D'UNE ADMINISTRATION CIBLEE DE PARTICULES MAGNETIQUES NANOMETRIQUES

Patent Applicant/Assignee:
TRITON BIOSYSTEMS INC, 200 Turnpike Road, Chelmsford, MA 01824, US, US (Residence), US (Nationality)
Inventor(s):
HANDY Erik S, 56 Milton Street, Arlington, MA 02474, US,
IVKOV Robert, 4 Goodwins Court, Marblehead, MA 01945, US,
ELLIS-BUSBY Diane, 875 Brockelman Road, Lancaster, MA 01523, US,
FOREMAN Allan, 2 Pawnee Lane, Epping, NH 03042, US,
BRAUNHUT Susan J, 47 MacArthur Road, Wellesley, MA 02482, US,
GWOST Douglas U, 1233 West Royal Oaks Drive, Shoreview, MN 55126, US,
ARDMAN Blair, 9 Orchid Circle, Marblehead, MA 01945, US,
JAHNGEN Edwin G E, 25 Hillside Road, Kingson, NH 03848, US,
Legal Representative:
LYNCH David W (agent), Altera Law Group LLC, Suite 100, 6500 City West Parkway, Minneapolis, MN 55344, US,

Patent and Priority Information (Country, Number, Date):
Patent: WO 200322360 A2-A3 20030320 (WO 0322360)
Application: WO 2002US23650 20020725 (PCT/WO US0223650)
Priority Application: US 2001307785 20010725; US 2002176950 20020618; US 2002200082 20020719
Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LU MC NL PT SE SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 25961

Main International Patent Class: **A61N-002/02**
International Patent Class: **A61N-001/40**
Fulltext Availability:
Detailed Description
Claims

English Abstract
Disclosed are therapeutic **methods** for the treatment of diseased, disease-causing, or undesirable tissue or material that involve the...

...of an alternating magnetic field to inductively heat the thermotherapeutic magnetic composition. Also disclosed are **methods** for administration of the thermotherapeutic magnetic composition. These therapeutic **methods** may be used where the predetermined target is associated with a disease, such as **cancer** , diseases of the immune **system** , pathogen-borne diseases, obesity, hormone-related diseases, Alzheimer's disease, disease precursor, and undesirable materials...

French Abstract
...a l'endroit ou la cible predeterminee est associee a la maladie, telle

qu'un **cancer** , des maladies du **systeme** immunitaire, des maladies transmises par des agents pathogenes, l'obesite, des maladies hormonales, la maladie...

Detailed Description

... thermotherapy, more specifically, to magnetic material compositions, devices for use with magnetic material compositions, and **methods** related thereto for thermotherapy via targeted delivery of nanoscale magnetic particles.

BACKGROUND

The time between...normal functions of healthy tissue or have other unwanted side effects.

One such disease is **cancer** . Despite considerable research effort and some success, **cancer** is still the second leading cause of death in the United States,

Claim

... more stressful course of therapy and may complicate patient compliance with prescribed therapies. Further, some **cancers** defy currently available treatment options, despite improvements in disease detection. Of the many fonns of **cancer** that still pose a medical challenge, prostate, breast, lung, and liver claim the vast majority of lives each year. Colorectal **cancer** ,
ovarian **cancer** , gastric **cancer** , leukemia, lymphoma, melanoma, and their
metastases may also be life-threatening. Conventional treatments for breast **cancer** , for example, typically include surgery followed by radiation and/or chemotherapy. These **techniques** are not always effective, and even if effective, they suffer from certain deficiencies. Surgical **procedures** range from removal of only the **tumor** (lumpectomy) to
complete removal of the breast. In early stage **cancer** , complete removal of the breast provides the best assurance against recurrence, but is disfiguring and difficult choice. Lumpectomy is less disfiguring, but is associated with a greater risk of **cancer** recurrence. **Radiation therapy** and chemotherapy are arduous and are not completely effective against recurrence. Treatment of pathogen-based...delayed, rather than halted.
For these reasons, it is desirable to provide improved and alternative techniques for treating disease. Such **techniques** should be less invasive and traumatic to the patient than the present **techniques** , and should only be effective locally at targeted sites, such as diseased tissue, pathogens, or other undesirable matter in the body. Preferably, the **techniques** should be capable of being performed in a single or very few treatment sessions (minimizing...

...a rapidly expanding type of therapy used for treating a variety of human diseases including **cancer** , for example. The FDA has approved a number of antibody-based **cancer** therapeutics. The ability to engineer antibodies, antibody fragments, and peptides with altered properties (e.g., antigen binding affinity, molecular architecture, specificity, valence, etc.) has enhanced their use in therapies. **Cancer** immunotherapeutics have made use of advances in the chimerization and humanization of mouse antibodies to...has a desired antigen binding affinity and specificity, and minimal immune response. The field of **cancer** immunotherapy makes use of markers that are overexpressed by **cancer** cells (relative to normal cells) or expressed only by **cancer** cells. The identification of such markers is ongoing and the choice of a ligand/marker...attaching them to an immunological cell

effector (bispecific antibodies). Although armed antibodies have shown potent **tumor** activity in clinical trials, they have also exhibited unacceptably high levels of toxicity to patients preferable.) These therapies often cause damage to non- **tumor** cells and present toxicity issues and delivery challenges. For example, **cancer** cells commonly shed surface-expressed antigens (targeted by immunotherapeutics) into the blood stream. Immune complexes...

...based therapies are diluted by interaction with these shed antigens instead of interacting with the **cancer** cells themselves, reducing the true delivered dose. Temperatures in a range from about 40 'C...destroyed via exposure to locally-high temperatures. Hyperthermia may hold promise as a treatment for **cancer** because it induces instantaneous **necrosis** (typically called "thertno-ablation") and/or a heat-shock response in cells (classical hyperthermia), leading to **cell death** via a series of biochemical changes within the cell. State-of-the-art **systems** that employ radiofrequency (RF) hyperthermia, such as annular phased array **systems** (APAS), attempt to tune E-field energy for regional heating of deep-seated **tumors** . Such **techniques** are limited by the heterogeneities of tissue electrical conductivity and that of highly perfused tissue...

...desired areas. These factors make selective heating of specific regions with such E-field dominant **systems** very difficult. Another strategy that utilizes RF hyperthermia requires surgical implantation of microwave or RF...for treatment of metastases because it requires knowledge of the precise location of the primary **tumor** . The seed implantation strategy is thus incapable of targeting undetected individual **cancer** cells or cell clusters not immediately adjacent to the primary **tumor** site. Clinical success of this strategy is hampered by problems with the targeted generation of heat at the desired **tumor** tissues.

SUMMARY OF THE INVENTION

Hyperthermia for treatment of disease using magnetic fluids exposed to... interest.

In view of the above, there is a need for a hyperthermia-based treatment **method** for diseased tissue that incorporates selective delivery of thennotherapeutic magnetic compositions to a diseased tissue. It is also desirable to have **methods** for treating diseased tissue in a safe and effective manner, with minimal invasion, and short...alternating magnetic field. It is yet another object of the present invention to provide a **method** that utilizes compositions of nanoscale magnetic materials and target-specific ligands in conjunction with a device that provides alternating magnetic fields to treat diseased tissue by **killing** diseased **cells** via hyperthermia. It is a further object of the present invention to provide **methods** for the treatment of diseased tissue, such as **cancer** , in a safe and effective manner, with minimal ...coating material for the particle; and a ligand that is selective to at least one **cancer** marker, and that can be bound to an uncoated portion of the particle, bound to...

...coating, or intercalated into the coating. The present invention also pertains to devices for treating **cancer** by interacting with magnetic particles targeted to **cancer** cells in a patient. Such a ...the gap being of sufficient size to receive a portion of the patient containing the **cancer** cells; and a power supply coupled to provide energy to the magnetic generator so that...

...at a frequency of about 1 kHz or more. The present invention further pertains to **methods** related to such magnetic material compositions and devices for the treatment of **cancer** . One such **method** includes the

administration to the patient of a magnetic material composition that includes at least one, single domain, magnetic particle attached to a **cancer** -cell specific ligand, and application of an alternating magnetic field to a region of the patient containing the **cancer** so as to inductively heat the magnetic material composition and **kill** the **cancer** **cells** . The therapeutic **methods** of the present invention provide for the treatment of diseased tissue, such as **cancer** , in a safe and effective manner, with minimal invasion, and short treatment periods. The above...in
connection with the accompanying drawings, in which:
Figure 1 schematically illustrates a thermotherapy treatment **system** according to an embodiment of the present invention;
Figure 2 schematically illustrates a thermotherapy treatment...diseased, disease-causing, or undesirable tissue or material, for use with magnetic material compositions, and **methods** for treating or removing the tissue or material utilizing such devices and magnetic material compositions. The therapeutic **methods** disclosed herein include the targeted delivery ...lipids, receptors, steroids, neurotransmitters, Cluster Designation/Differentiation (CD) markers, imprinted polymers, and the like. The **methods** for treating diseased tissue disclosed herein include administering to a patient the bioprobes suspended in...to a specific location within a patient 105 by a magnetic circuit 102. The therapeutic **methods** of the present invention may be performed following a determination of the presence of disease...of the patient. For example, the disease material may be any one or combination of **cancers** and **cancerous** tissue, a pathogenic infection (viral, bacterial or multicellular parasitic), toxin, or any pathogen-like material...

...diagnosis does not form part of the invention and may be performed using any standard **method** . However, the present invention, or aspects thereof, may be amenable to a diagnostic function alone or in conjunction with another **method** or apparatus. Such a diagnostic function would be performed by using a suitable technology or **technique** to interrogate the ...the patient. Both the location and concentration of bioprobes may be determined using an existing **technique** such as magnetic
resonance imaging, or another diagnostic **technique** can be established and performed using a suitable magnetometer, such as a Superconducting Quantum Interference...the disease material 214. In the illustrated case, the bioprobes 210 are selective to breast **cancer** . The bioprobes 210 become excited by the interacting applied AMF and are inductively heated to...

...example, heat generated in the bioprobes 210 may pass to the cells, thereby causing the **cells** to **die** .
Furthermore, the poles 204 may be formed from pieces whose gap is adjustable, so as...the -3dBc points of the output of a square law crystal detector. Because this measurement **technique** is cumbersome in this application, we use an alternate definition of pulse width. For the ...strength can be increased, and thereby reducing the treatment time and increasing the efficacy. One **method** of confining the high peak amplitude ANW to treatment area 205 is by defining the...likelihood that they will need to be removed prior to treatment, thereby avoiding invasive medical **procedures** . Concentrating the field permits the treatment of large volumes within the chest or trunk with...composition and location within the body, possesses unique heating and cooling capacities. For example, a **tumor** located within a region that is poorly ...located within a relatively insulating region may require a lower Curie temperature material than a **tumor** that is located near a major blood vessel. Targets that are in the bloodstream will...material may

also serve to facilitate transport of the bioprobe 690 into a cell, a **process** known as transfection. Such coating materials, known as transfection agents, include ...The association of a ligand or ligands with the bioprobes 690 allows for targeting of **cancer** or disease markers on cells. It also allows for targeting biological matter in the patient...immunogenic response. In another embodiment, the ligand 640 may be designed to target a specific **cancer** cell marker or markers. The particular **cancer** cell marker and ...640 may be specific to, but not limited to, the type and location of the **cancer** such as, for example, **tumors** , metastatic **cancer** , minimal residual disease and the like. The ligand(s) 640 may have an affinity for the **cancer** marker or markers of interest. The **cancer** marker or markers may be selected such that they represent a viable target on the **cancer** cells of interest. The preferred **cancer** marker may be expressed on the surface of the **cancer** cells and is preferably present in very low amounts or not at all in non-nal cells. The preferred **cancer** marker may not be readily shed from the surface, or if shed, the ligand on...mechanisms. When a sufficient amount of heat is transferred by the bioprobes 890 to the **cell** , the **cell dies** by **necrosis** , **apoptosis** or another mechanism. The choice of a marker (antigen) 850 may be important to therapy utilizing bioprobes. For breast **cancer** and its metastases, a specific marker or markers may be chosen from cell surface markers...

...for example, members of the MUCtype mucin family, an epithelial growth factor (EGFR) receptor, a **carcinoembryonic** antigen (CEA), a human **carcinoma** antigen, a vascular endothelial growth factor (VEGF) antigen, a melanoma antigen (MAGE) gene, family antigen...

...Fn antigen, a hormone receptor, growth factor receptors, a cluster designation/differentiation (CD) antigen, a **tumor** suppressor gene, a cell cycle regulator, an oncogene, an oncogene receptor, a proliferation marker, an adhesion molecule, a proteinase involved in degradation of extracellular matrix, a **malignant** transformation related factor, an **apoptosis** related factor, a human **carcinoma** antigen, glycoprotein antigens, DF3, 4F2, MGFM antigens, breast **tumor** antigen CA 15-3, calponin, cathepsin, CD 31 antigen, proliferating cell nuclear antigen 10 (PC 10), and pS2. For other forrns of **cancer** and their metastases, a specific marker or markers may be chosen from cell surface markers...

...for example, a member of vascular endothelial growth factor receptor (VEGFR) family, a member of **carcinoembryonic** antigen (CEA) family, a type of anti-idiotypic mAB, a type of ganglioside mimic, a...of a cellular adhesion molecule, a member of MUC-type mucin family, a type of **cancer** antigen (CA), a type of a matrix metalloproteinase, a type of glycoprotein antigen, a type of melanoma associated antigen (MAA), a proteolytic enzyme, a calmodulin, a member of **tumor necrosis** factor (TNTF) receptor family, a type of angiogenesis Marker, a melanoma antigen recognized by T...

...antigen encoding gene (MAGE) family, a prostate membrane specific antigen (PMSA), a small cell lung **carcinoma** antigen (SCLCA), a T/Tn antigen, a hormone receptor, a **tumor** suppressor gene antigen, a cell cycle regulator antigen, an oncogene antigen, an oncogene receptor antigen, a proliferation marker, a proteinase involved in degradation of extracellular matrix, a **malignant** transfon-nation related factor, an **apoptosis** -related factor, a type of human **carcinoma** antigen. In an embodiment of the invention, a bioprobe may include ligand(s) targeting the...substrate adhesion. WC-1 is highly expressed in many human adenocarcinomas, including 80% of breast **cancers** , and is associated with poor prognosis. Mucin (MUC-1 and

MUC-2) expression is associated with **tumor** invasiveness. WC- 1 and WC-2 expression is associated with invasive ductive **carcinoma** of the breast. WC-1 is also present at high levels on many mylomas. Different tissues/cells produce differing glycofon-ns of WC Glycosylation of NIUC-I in **malignant** cells is often altered compared to normal tissue. NIUC-1 is considered a truly **tumor** specific antigen, although it is also found on normal cells, its aberrant glycosylation on **tumors** creates new epitopes for targeting. The extracellular domain of WC-1 may be shed into ...cell surface.

Overexpression of growth factor receptors such as the EGFR family is indicated in **tumors** and has been associated with increased cell resistance to the cytotoxic effects of macrophages and cytotoxic factors, such as TNF (**tumor** **necrosis** factor), which can lead to **tumor** growth. The protein encoded by the Her-1/neu gene is a 170,000 Dalton... unshed remainder of the Her-2 expressed on the surface of the cell. For ovarian **cancers** and their metastases, a specific marker or markers may be chosen from cell surface markers...

...example, one of ERBB2 (Her-2) antigen and CD64 antigen. For ovarian and/or gastric **cancers** and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, a polymorphic epithelial mucin (PEM). For ovarian **cancers** and their metastases, a specific marker or markers may be chosen from cell surface markers

such as, for example, one of **cancer** antigen 125 (CA125) or matrix metalloproteinase 2 (NEqP-2). For gastric **cancers** and their metastases, a specific marker or markers may be chosen from cell surface markers...

...for example, one of CA19-9 antigen and CA242 antigen.

For non-small-cell lung **cancer** (NSCLC), colorectal **cancer** (CRQ and their

metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, vascular endothelial growth factor receptor (VEGFR), antiidiotypic mAb, and **carcinoernbryonic** antigen (CEA) mimic. For at least one of small-cell lung **cancer** (SCLQ, **malignant** melanoma, and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, anti-idiotypic mAB or GD3 ganglioside mimic. For melanoma **cancers** and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, a melanoma associated antigen (MAA). For small cell lung **cancers** and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, a small cell lung **carcinoma** antigen (SCLCA).

For colorectal **cancer** (CRQ and/or locally advanced or metastatic head and/or neck **cancer** , a specific marker or markers may be chosen from cell surface markers such as, for example, epidermal growth factor receptor (EGFR). For Duke's colorectal **cancer** (CRQ and its metastases, a specific marker or markers may be chosen from cell surface...may be chosen from cell surface markers such as, for example, CD33 antigen. For prostate **cancers** and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, prostate membrane specific antigen (PMSA). For **carcinomatous** meningitis and their metastases, a specific marker or markers may be chosen from cell surface...glycoprotein, for example, HTAFGI (human milk fat globulin) antigen.

For lung, ovarian, colon, and melanoma **cancers** and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, B7-HI protein. For colon, breast, lung, stomach, cervix, and uterine **cancers** and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, TRAEL Receptor-1 protein, a member of the **tumor** **necrosis** factor receptor

family of proteins. For ovarian, pancreatic, non-small cell lung, breast, and head and neck **cancers** and their metastases, a specific marker or markers may be chosen from cell surface markers such as, for example, EGFR (epidermal growth factor receptor). For anti-angiogenesis targeting of **tumor** blood supply, a specific marker or markers may be chosen from cell surface markers such...

...marker specific to endothelial cells of growing blood vessels. For targeting of colon and bladder **cancer** and their metastases, a specific marker or markers may be chosen from cell surface markers...the external environment to the nucleus. A mutated fonn of RAS is found in many **cancers** .

In another embodiment, the ligand 640 may be targeted to a predetermined target associated with a disease of the patient's immune **system** . The particular target and ligand(s) 640 may be specific to, but not limited to s immune **system** . The ligand(s) 640 may have an affinity for a target associated with a disease of the patient's immune **system** such as, for example, a protein, a cytokine, a chemokine, an infectious organism, and the like. For diseases of the patient's immune **system** , a specific marker or markers may be chosen from cell surface markers. The targeted cells may be T or B cells of the immune **system** . For rheumatoid arthritis, a specific marker or markers may be chosen from cell surface markers such as, for example, one of CD52 antigen, **tumor necrosis** factor (TNF), ...represent a viable target on T cells or B cells of the patient's immune **system** .

For reaction to a transplanted organ, the ligand(s) 640 may be chosen to target...another embodiment, the ligand 640 may be targeted to a predetermined

target associated with non- **cancerous** diseased tissue. The particular target and ligand(s) 640 may be specific to, but not limited to, a particular non- **cancerous** diseased tissue. The ligand(s) 640 may have an affinity for a biological molecule associated with the non- **cancerous** diseased tissue. The ligand(s) 640 may have an affinity for a cell marker or markers associated with the non- **cancerous** diseased tissue. For non- **cancerous** diseased ...may be chosen to be a predetermined target such as, for example, one of non- **cancerous** diseased deposits and precursor deposits. For Alzheimer's disease, a predeten-nined target may be...and returning it to the body. The bioprobes may be introduced into the vascular circulating **system** or into the blood circulating outside of the body. For example, to target bioprobe/target...

...returning the blood to the body. The bioprobes may be introduced into the vascular circulating **system** , the blood circulating outside of the body, or the blood serum or plasma ...using either treatment within the patient (intracorporeal) and treatment external to the patient (extracorporeal).

A **method** of administering the bioprobes 890 to the desired area for treatment and the dosage may...material. The size range of the bioprobes 890 allows for microfiltration for sterilization. An administration **method** may be, for example, wash, lavage, as a rinse with sponge, or other surgical cloth as a perisurgical

administration **technique** . Other **methods** of administration may include intravascular injection, intravenous injection, intraperitoneal injection, subcutaneous injection, and intramuscular injection...magnetic nature of the bioprobes. Assisted delivery may depend on the location of the targeted **cancer** . The bioprobes may also be delivered to the patient using other **methods** . For example, the bioprobes may be administered to the patient orally, or may be administered rectally.

Another **method** of administering the bioprobes 890 to the desired area for treatment may include administering the...administered separately and

combine with the streptavidin fusion protein in the patient's body. Another **method** of administering the bioprobes 890 to the desired area for treatment may include administering the...body to form a targeted bioprobe for therapy.
Once administered by any of the above **methods** , the movement of the bioprobes can be influenced by an AW with a DC offset...field conditions, field amplitude and pulse characteristics, to levels that heats the bioprobes sufficiently to **kill** targeted **cells** without excessive peripheral tissue heating. Thus, under pulsed conditions, peak magnetic fields of up to...

...I may be coated with a
biocompatible polymer according to a following embodiment of the **procedure** . Poly (methacrylic acid-co-hydroxyethylmethacrylate) as a biocompatible coating material
for bioprobes may be synthesized from methacrylic acid and hydroxyethyl methacrylate using **free - radical** polymerization in the presence of ...may react with the polymer coating. Conjugation of the antibodies to the
nanoparticles using this **technique** may proceed in the following manner. The antibody may be covalently conjugated to 4-[p...coordinate covalent linkages. Example 4: Efficacy of bioprobes: In vitro trials with MCF-7 breast **cancer** cells.
The bioprobes included 50-nm, Fe3O4 particles surrounded by a dextran shell, to which...the media in all samples monitored in situ using silicon dioxide temperature probes resistant to **electromagnetic** (EM) **fields** obtained from FISO Technologies Inc., Ste-Foy, Quebec, Canada. A series of water bath tests...

...tests was to obtain "positive control" data on the effects of hyperthen-nia on the **cancer** cell cultures and to aid in the interpretation of experiments using the bioprobe **system** . Exposing cellular culture media to an alternating magnetic field, without the presence of the bioprobes...

...heating of the media containing cells not receiving the treatment. Table 1 lists results of **cell death** fraction as a function of exposed temperature and exposure time. The MCF-7 cell line...Table 2. In the targeted sample, 91% 1 5% (n = 7) of the MCF-7 **cells** were **killed** . Of the **cells killed** , about 70% **cells** were be lysed by the treatment, as measured by spectrophotornetric analysis of cytoplasmic lactate dehydrogenase (LDH), an enzyme produced by living cells. The remaining approximately 20% underwent **apoptosis** , as measured using a commercial fluorescent **apoptosis** -staining assay. The kill rates for the targeted cells are significantly higher than baseline death and **apoptotic** rates of 4% 1 1% in all controls (Table 2). Control groups include: 1) cells cells. Higher than normal **cell death** occurs only when the AW is applied after the bioprobes have attached to a cell...

...1 5
Example 5: Selectivity of bioprobes: In vitro trials with SK-CO-1 colon **cancer** cells. For the in vitro studies, the MCF-7 human breast **cancer** cells were chosen because their Her-2 expression was sufficient for detection, but was not ...high amounts as is the case with the more aggressive NMA-NM-231 (human breast **carcinoma** cells known to significantly over express Her-2) cell line. As a control, cultures of...

...chosen to provide a reasonable model to challenge the effectiveness and selectivity of the bioprobe **system** . To investigate the selectivity of the bioprobes, SK-CO-1 human colon **cancer** cells known to be Her-2

negative were treated in the same manner. For this group, rates of **apoptosis** were 13% for ...CO-I cells when used with any or all components of the invention, demonstrating selective **tumoricidal** activity for the MCF-7 breast cells. TABLE 3: In Vitro Results with SK-CO ...AMF 91 1 5 (N=7)

Example 6: Bioprobe targeting MUC- I receptor in breast **cancer** . The target on the breast **cancer** cells may be MUC-1 (Human epithelial mucin, CD277) marker. MUC- I is highly expressed in many human adenocarcinomas including 80% of breast **cancers** and is associated with poor prognosis. The ligand on the bioprobe may be an antibody...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3, available from Pierce, Rockford, EL, may be used with and proof of **killing** of **cancer** **cells** above the level of the controls. The targeted bioprobes may be further refined by producing...

...such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the **tumor** targeting and potency. The use of fragments, humanized antibody, or peptides creates a ligand that avoids immunogenic response in humans.

The MUC-I receptor is considered a truly **tumor** specific antigen because

although it is on normal cells, its aberrant glycosylation on **tumors** creates new epitopes for targeting. The extracellular domain of MUC-1 is shed into the NWC-1 which remains on the **cancer** cell after a portion of the extracellular domain is shed. This choice of ligand allows the bioprobe to specifically and selectively target breast **cancer** cells.

Example 7: Bioprobe targeting non-small cell lung **cancer** , VEGFR. The bioprobes may be designed to target many different kinds of **cancer** . A target on non-small cell lung **cancer** cells may be VEGFR (vascular endothelial growth factor receptor). marker. VEGFR may also be a viable target for many other

types of solid **cancer** **tumors** . VEGFR has been implicated in **tumor** induced

angiogenesis, or blood vessel growth which has been shown to play an important role in the development of many solid **tumors** . Angiogenesis is induced by solid **tumors** to provide nutrients to enable growth. The bioprobe therapy may target **tumor** -induced angiogenesis and prevent the blood vessel growth needed by a **tumor** . Since there is little, if any, blood vessel growth in a healthy adult, the targeting...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3, available from Pierce, Rockford, EL, may be used with...

...containing, 50 nm dextran coated particle. In vitro studies demonstrate dose response and proof of **killing** of **cancer** **cells** above the level of the controls. The targeted bioprobes may be further refined by producing...such as antigen binding affinity, molecular architecture, and specific receptor epitope targeting to enhance the **tumor** targeting and potency. The use of fragments, humanized antibody, or peptides creates a ligand that avoids immunogenic response in humans. Example 8: Bioprobe targeting immune **system** conditions, CD52. A target rheumatoid arthritis may be cluster designation/differentiation CD52 antigen. CD52 may also be a viable target for other immune **system** diseases such as multiple sclerosis. For diseases of the patient's immune **system** , the bioprobe therapy may target the T or B cells of the immune **system** . Typically these cells are involved in an immune response. T lymphocytes play a central role...self-tolerance.

The damage which occurs in these autoimmune diseases is analogous to the rejection **process** which occurs after transplantation of a foreign

organ. Adults also have active mechanisms for maintaining...

...which are controlled by a complex symphony of interactions between different parts of the immune **system** . Bioprobes with antibodies to CD52 can destroy lymphocytes and disrupt the immune **system** at the most fundamental level. Unlike **cancer** therapy, it may not be the aim to kill all the target cells, but just hope was that when the immune **system** regenerated over the subsequent months, the vicious cycle of tissue injury, resulting in fresh immune...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3, available from Pierce, Rockford, EL, may be used with...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3' available from Pierce, Rockford, EL, may be used with... called hepatitis B virus (HBV), can cause lifelong infection, cirrhosis (scarring) of the liver, liver **cancer** , liver failure, and death. In this particular embodiment of bioprobe therapy, the target is the several different **techniques** . A linking agent, such as BS3 , available from Pierce, Rockford, EL, may be used with...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3, available from Pierce, Rockford, EL, may be used with...and is transmitted via blood sucking Reduvid bugs. Acute phase symptoms include fever, malaise, lymphatic **system** abnormalities, and enlargement of the spleen and liver. Fatal pathologies include parasitic infection of the...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3 , available ...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3, available from Pierce, Rockford, EL, may be used with...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3' available from Pierce, Rockford, EL, may be used with...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3, available from Pierce, Rockford, EL, may be used with...transplanted into the patient. This application is similar to diseases of the patient's immune **system** . The bioprobe therapy may target the T or B cells of the immune **system** . Typically these cells are involved in an immune response. T lymphocytes play a central role... invaders from its own tissues is becoming known. The damage which occurs in the rejection **process** which occurs after transplantation of a foreign
organ is analogous to autoimmune diseases. Bioprobes which can destroy lymphocytes and disrupt the immune **system** at the most fundamental level can be used to treat organ transplant rejection. Unlike **cancer** therapy, it may not be the aim to kill all the target cells, but just **system** regenerated over the subsequent months, the vicious cycle of tissue injury, resulting in fresh immune...may be linked to the magnetic particle or the dextran coating through several
3
different **techniques** . A linking agent, such as BS , available from Pierce, Rockford, EL, may be used with...specific receptor epitope targeting to enhance the targeting and potency. Example 17: Bioprobe targeting non- **cancerous** diseased tissue. The bioprobes may be designed to target cells, tissue, or biological molecules related to a non- **cancerous** disease. One particular example of a target related to a non- **cancerous** disease is B protein and its deposits a

ssociated with Alzheimer' ...s
disease. Bioprobes designed to specifically bond to lipoproteins that

transport arnyloids in the vascular **system** can be used to target, denature, or otherwise deactivate these lipoproteins when exposed to AUT ...amyloid B protein, associated with Alzheimer's disease, as they are transported in the vascular **system** or after they are deposited in the brain, can be used to target, denature or...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3 , available from Pierce, Rockford, EL, may be used with...portion may be linked to the magnetic particle or the dextran coating through several different **techniques** . A linking agent, such as BS3 , available from Pierce, Rockford,]IL, may be used with...In another example, overproduction of the female hormone estrogen may
increase the risk for breast **cancer** . The number of new **cancer** cells increases proportionally to the amount of overexpression by the estrogen precursor--and these effects...and returning it to the body. The bioprobes may be introduced into the vascular circulating **system** or into the blood circulating outside of the body. For example, to target bioprobe/target...returning the blood to the body. The bioprobes may be introduced into the vascular circulating **system** , the blood circulating outside of the body, or the blood serum or plasma after it...least one of the duty cycle, the PRF, the magnitude of the magnetic field. This **process** continues until the treatment is completed. Example 23: Preliminary temperature monitoring to optimize treatment. Using... mammals. As noted above, the present invention is applicable to a magnetic material composition, a **system** and **method** of thermotherapy for the treatment of at least **cancerous** tissue, non- **cancerous** diseased tissue and undesirable tissue. The present invention should not be considered limited to the...

...aspects of the invention as fairly set out in the attached claims. Various modifications, equivalent **processes** , as well as numerous structures to which the present invention may be applicable will be...of the present specification. The claims are intended to cover such modifications and devices.
. A **method** for treating a patient, comprising:
administering a magnetic material composition to at least a portion...

...composition combined with the predetermined target to inductively heat the magnetic material composition.

2 The **method** as recited in claim 1, wherein the predetermined target is
associated with a **cancer** .

3 The **method** as recited in claim 2, wherein the predetermined target is one of a) a member of vascular endothelial growth factor receptor (VEGFR) family, b) a member of **carcinoembryo**nic antigen (CEA) family, c) an anti-idiotypic mAB, d) a ganglioside mimic, e) a member...

...g) a cellular adhesion molecule, h) a member of MUC-type mucin family, i) a **cancer** antigen (CA), j) a matrix metalloproteinase, k) ...melanoma associated antigen (MAA), m) a proteolytic enzyme, n) a calmodulin, o) a member of **tumor** **necrosis** factor (TN-F) receptor family, p) a angiogenic marker, q) a
melanoma antigen recognized by...

...gene (MAGE) family, s) a prostate membrane specific antigen (PMSA), t) a small cell lung **carcinoma** antigen (SCLCA), u) a proliferating cell nuclear antigen 10 (PC10), v) pS2, w) a T/Tn antigen, x) a hormone receptor, y) a **tumor** suppressor gene antigen, z) a cell cycle regulator

antigen, aa) an oncogene ...cc) a proliferation marker, dd) a proteinase involved in degradation of extracellular matrix, ee) a **malignant** transformation related factor, ff) an **apoptosis** -related factor gg) a human **carcinoma** antigen and any combination of a) through gg).
. The **method** as recited in claim 3 wherein the ganglioside mimic is GD3.

5 The **method** as recited in claim 3, wherein the epidermal growth factor receptor (EFGR) includes at least...

...of Her-1, Her-2, Her-3, Her-4 and any combination thereof.

6 The **method** as recited in claim 3, wherein the cluster designation/differentiation ...antigen, CD52 antigen, CD64 antigen, CD31 antigen, CD33 antigen and any
combination thereof.

7 The **method** as recited in claim 3, wherein the cellular adhesion molecule is epithelial cellular adhesion molecule (Ep-CAM) antigen.
8 The **method** as recited in claim 3, wherein the WC-type mucin includes at least one of polymorphic epithelial mucin (PEM), WC-1 and any combination thereof.

9 The **method** as recited in claim 3, wherein the **cancer** antigen is at least one of CA125, CA19-9, CA15-3 (breast **tumor** antigen), CA242 and any
combination thereof.

10 The **method** as recited in claim 3, wherein the matrix metalloproteinase is matrix metalloproteinase 2 (MW-2).

11 The **method** as recited in claim 3, wherein the glycoprotein antigen is
epithelial associated glycoprotein (HWGI antigen).

12 The **method** as recited in claim 3, wherein the proteolytic enzyme is at least one of cathepsin...

...cathepsin D, cathepsin L, cathepsin B, cathepsin C, cathepsin S and any combination thereof. . The **method** as recited in claim 3, wherein the calmodulin is calponin.
14 The **method** as recited in claim 3, wherein the **tumor necrosis** factor
receptor is a TRAEL Receptor- 1 protein.

15 The **method** as recited in claim 3, wherein the angiogenic marker is at
least one of vascular endothelial growth factor receptor (VEGFR), Integrin avP3 and any combination thereof.

16 The **method** as recited in claim 2, further comprising:
applying an alternating magnetic field to a region of the patient containing the **cancer** , wherein the alternating magnetic field is applied using a device
comprising:
a magnetic generator having of sufficient size to receive a portion of the patient
containing the **cancer** cells; and
a power supply coupled to provide energy to the magnetic generator so that...

...the two poles alternates at a frequency of about I kHz or more.
17 A **method** according to claim 16, wherein the magnetic material composition inductively heats to a sufficient temperature to induce **cancer cell death** via **necrosis** , **apoptosis** or another mechanism.

18 A **method** according to claim 16, further comprising applying the alternating magnetic field to a region of the patient containing **cancer** tissue and to a region of the patient adjacent the region containing the **cancer** tissue.

19 A **method** according to claim 16, further comprising applying a static magnetic field to a region of the patient containing **cancer** tissue to aid in localizing the magnetic material composition to the region containing the **cancer** tissue.
. A **method** according to claim 16, further comprising monitoring at least one physical characteristic of at least one of **cancer** tissue and non- **cancerous** disease material.

21 A **method** according to claim 16, further comprising pulsing the alternating magnetic field while applying the alternating...two pulses of the alternating magnetic field while applying the alternating magnetic field.
22 A **method** according to claim 16, wherein the alternating magnetic field is applied to the patient over...

...and applying at least two pulses of alternating magnetic field to the patient.

23 The **method** as recited in claim 16, wherein administering the magnetic material composition further comprises administering the material to a specific area of a patient's body via an administration **technique** being at least one of injecting the composition intravenously, injecting the composition intraperitoneally, injecting the...

...perisurgically, inhaling the composition, orally ingesting the composition and rectally inserting the composition.

24 A **method** according to claim 16, wherein a magnetic field generator generates the alternating magnetic field and A **method** according to claim 16, wherein applying the alternating
magnetic field includes modulating the alternating magnetic...

...triangular envelope, a square wave envelope, a trapezoidal envelope and a sawtooth envelope.
26 A **method** according to claim 16, wherein the magnetic material composition includes a biocompatible coating material covering at least part of the magnetic particle.

27 The **method** as recited in claim 1, wherein the predetermined target is
an antigen associated with a disease of a patient's immune **system** , the disease being one of rheumatoid arthritis, vasculitis and multiple sclerosis.

28 The **method** as recited in claim 27, wherein the predetermined target is a cluster designation/differentiation antigen.

29 The **method** as recited in claim 1, wherein the predetermined target is

a virus, the predetermined target...

...Epstein-Barr virus (EBV), Hepatitis virus, human immunodeficiency virus (HIV) and Herpes virus.

30 The **method** as recited in claim 1, wherein the predetermined target is
one of an undesirable materialLDL) and clotted blood.

31 The **method** as recited in claim 1, wherein the predetermined target is
associated with a reaction to...

...antigen, CD4 antigen, CD 8 antigen, CD18 antigen, CD52 antigen and CD154 antigen.

32 The **method** as recited in claim 1, wherein the predetermined target is
non- **cancerous** disease material and associated with non- **cancerous** disease material, the predeten-nined target being one of a non- **cancerous** disease deposit and a noncancerous disease precursor deposit.

33 The **method** as ...wherein the predetermined target is one of an arnyloid protein and an apolipoprotein.

34 The **method** as recited in claim 32, wherein the predetermined target is associated with an HIV infected cell and wherein the predetermined target is CTLA4 protein.

35 The **method** as recited in claim 1, wherein administering the magnetic material composition further comprises administering the material to a specific area of a patient's body via an administration **technique** being at least one of injecting the composition intravenously, injecting the composition ...perisurgically, inhaling the composition, orally ingesting the composition and rectally inserting the composition.

36 The **method** as recited in claim 1, wherein administering the magnetic material composition further comprises:
administering the...

...and
combining the ligand with the magnetic particle in the patient's body.

37 The **method** as recited in claim 36, wherein administering the magnetic material composition further comprises:
administering the...magnetic particle by uniting the binder and receptor in the patient's body.

38 The **method** as recited in claim 37, wherein the binder is biotinylated
and the receptor is an avidin.

39 The **method** as recited in claim 36, wherein administering the magnetic material composition further comprises:
administering a the magnetic particle with the ligand in the patient's body.

40 The **method** as recited in claim 1, wherein applying the alternating magnetic field includes applying the alternating magnetic field when the magnetic material composition is outside the patient's body.

41 The **method** as recited in claim 40, wherein administering comprises

providing the magnetic material composition within the...

...magnetic material composition from the patient's body after attaching to the target.

42 The **method** recited in claim 40, further comprising removing, after applying the alternating magnetic field, the magnetic...and prior to returning the extracted bodily materials to the patient's body.

43 The **method** as recited in claim 40, wherein administering comprises extracorporeal administration of the magnetic material composition into a patient's bodily materials.

. The **method** as recited in claim 43, wherein extracorporeal administration comprises:

extracting bodily materials from the patient...

...extracted bodily materials; and

exposing the bodily materials to the alternating magnetic field.

45 The **method** as recited in claim 40, wherein the extracted bodily materials are returned to the patient's body after exposure to the alternating magnetic field.

46 The **method** as recited in claim 40, wherein the extracted bodily materials are returned to the patient's body before exposure to the magnetic field.

47 The **method** as recited in claim 40, wherein the extracted bodily materials include at least one of blood and blood products extracted from the patient.

48 The **method** as recited in claim 1, further comprising detecting at least one location of accumulation of the magnetic material composition within the patient's body.

49 The **method** as recited in claim 48, wherein detecting the at least one

location of accumulation ...composition to the patient and prior to application of the alternating magnetic field.

50 The **method** as recited in claim 49, further comprising determining an amount of the magnetic material composition...

...or indirect detection of the particles via at least one of NMI and SQUID.

. The **method** as recited in claim 50, wherein the determined amount of magnetic material composition taken up...calculate a condition and duration of the alternating magnetic field to be applied.

52 The **method** as recited in claim 1, further comprising inducing a desired pathological effect by inductively heating the magnetic material causing one of **necrosis** , **apoptosis** and deactivation of disease material in a targeted region.

53 The **method** as recited in claim 1, further comprising applying the alternating magnetic field to a region...

...a region of the patient adjacent to the region containing the predetermined target.

54 The **method** as recited in claim 1, further comprising localizing ... alternating magnetic field to the region of the patient containing the predetermined target.

55 The **method** as recited in claim 1, further comprising monitoring at least one physical characteristic of a...

...at least one physical characteristic being one of tissue temperature and tissue impedance.

56 The **method** as recited in claim 1, wherein the alternating magnetic field is applied to the patient...applying at least two pulses of the alternating magnetic field to the patient.

57 The **method** as recited in claim 1, further comprising generating with a magnetic field generator an alternating...

...about 10 Oersteds (Oe) to about 10,000 Oe proximate the magnetic field generator.

. The **method** as recited in claim 1, wherein applying the alternating magnetic field comprises modulating the alternating...triangular envelope, a square wave envelope, a trapezoidal envelope and a sawtooth envelope.

59 The **method** as recited in claim 1, wherein applying the alternating magnetic field comprises:
providing and positioning...

...auxiliary coils with a d.c. component to influence the magnetic field pattern.

60 The **method** as recited in claim 59, wherein the d.c. component has a magnitude sufficient to...a unidirectional nature with varying intensity and a time-averaged, non-zero value.

61 The **method** as recited in claim 59, further comprising driving the auxiliary coils in series with the primary coil.

62 The **method** as recited in claim 59, further comprising driving the auxiliary coils in parallel with the primary coil.

63 The **method** as recited in claim 59, further comprising electrically matching the auxiliary coils with a phase from the alternating magnetic field.

64 The **method** as recited in claim 59, further comprising influencing the
magnetic field pattern to separate the...

...associated with the magnetic field, so as to reduce dielectric heating of the patient.

. The **method** as recited in claim 1, wherein applying the alternating magnetic field further comprises providing a primary coil as a core-less coil.

66 The **method** as recited in claim 1, wherein applying the alternating magnetic field comprises applying the alternating magnetic field using only a primary coil.

67 The **method** as recited in claim 1, ...magnetic field generator and

further comprises cooling the magnetic field generator with liquid.
68 The **method** as recited in claim 67, further comprising cooling a core of the magnetic field generator with liquid.

69 The **method** as recited in claim 1, wherein applying the alternating magnetic field includes driving a primary...

...coils disposed proximate respective first and second pole pieces of a magnetic core.

70 The **method** as recited in claim 1, wherein ...composition of claim 71, wherein the disease
material marker is specific to one of breast **cancer** , metastatic **cancer** related to breast **cancer** and primary **cancer** of the breast.

74 A magnetic particle composition of claim 71, wherein the biocompatible coating...a) a member of vascular endothelial growth factor receptor (VEGFR) family, b) a member of **carcinoembryonic** antigen (CEA) family, c) an anti-idiotypic rnAB, d) a ganglioside mimic, e) a member...

...g) a cellular adhesion molecule, h) a member of MUC-type mucin family, i) a **cancer** antigen (CA), j) ...melanoma associated antigen (MAA), m) a proteolytic enzyme, n) a calmodulin, o) a member of **tumor necrosis** factor (TNF) receptor family, p) a angiogenic marker, q) a melanoma antigen recognized by T...

...gene (MAGE) family, s) a prostate membrane specific antigen (PMSA), t) a small cell lung **carcinoma** antigen (SCLCA), u) a proliferating cell nuclear antigen 10 (PCIO), v) pS2, w) a T/Tn antigen, x) a hormone receptor, y) a **tumor** suppressor gene antigen, z) a cell cycle regulator antigen, ...cc) a proliferation marker, dd) a proteinase involved in degradation of extracellular matrix, ee) a **malignant** transformation related factor, ff) an **apoptosis** -related factor gg) a human **carcinoma** antigen, hh) DF3 antigen, ii) 4F2 antigen, J) WGM antigen and any combination of a...

US 20030032995A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2003/0032995 A1

Handy et al. (43) Pub. Date: Feb. 13, 2003

(54) THERMOTHERAPY VIA TARGETED DELIVERY OF NANOSCALE MAGNETIC PARTICLES

(75) Inventors: **Erik S. Handy**, Arlington, MA (US); **Robert Ivkov**, Marblehead, MA (US); **Diane Ellis-Busby**, Lancaster, MA (US); **Allan Foreman**, Epping, NH (US); **Susan J. Braunhut**, Wellesley, MA (US); **Douglas U. Gwost**, Shoreview, MN (US); **Blair Ardman**, Marblehead, MA (US)

Correspondence Address:
ALTERA LAW GROUP, LLC
6500 CITY WEST PARKWAY
SUITE 100
MINNEAPOLIS, MN 55344-7704 (US)

(73) Assignee: **Triton Biosystems, Inc.**, Chelmsford, MA

(21) Appl. No.: **10/200,082**

(22) Filed: **Jul. 19, 2002**

Related U.S. Application Data

(60) Provisional application No. 60/307,785, filed on Jul. 25, 2001.

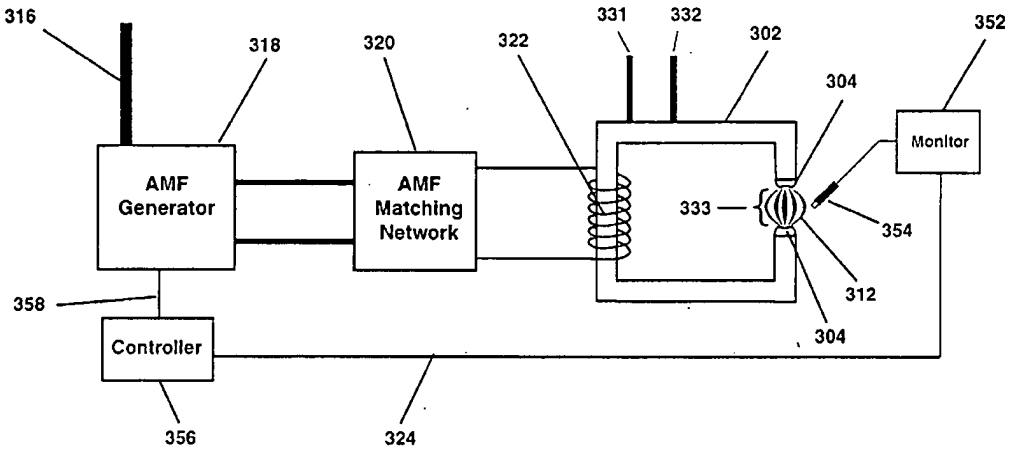
Publication Classification

(51) **Int. Cl.**[7] **A61F 7/00**; A61F 7/12; A61F 2/00

(52) **U.S. Cl.** **607/103**; 607/96

(57) **ABSTRACT**

Disclosed are therapeutic methods for the treatment of disease material involving administration of a thermotherapeutic magnetic composition, which contains single-domain magnetic particles attached to a target-specific ligand, to a patient and application of an alternating magnetic field to inductively heat the thermotherapeutic magnetic composition. Also disclosed are methods of administering the thermotherapeutic magnetic material composition. The thermotherapeutic methods may be used where the predetermined target is associated with diseases, such as cancer, diseases of the immune system, and pathogen-borne diseases, and undesirable targets, such as toxins, reactions associated with organ transplants, hormone-related diseases, and non-cancerous diseased cells or tissue.



25/3,K/10 (Item 10 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00240849
Method **and apparatus for selective irradiation of biological materials.**
Verfahren und Vorrichtung zur selektiven Bestrahlung von biologischen Materialien.
Methode **et appareil pour irradiation selective de materiaux biologiques.**
PATENT ASSIGNEE:
Mills, Randell L., (745290), R.D. 2, Cochranville Pennsylvania 19330, (US), (applicant designated states: DE;FR;GB)
INVENTOR:
Mills, Randell L., R.D. 2, Cochranville Pennsylvania 19330, (US)
LEGAL REPRESENTATIVE:
Patentanwalte Beetz sen. - Beetz jun. Timpe - Siegfried - Schmitt-Fumian (100711), Steinsdorfstrasse 10, W-8000 Munchen 22, (DE)
PATENT (CC, No, Kind, Date): EP 240990 A2 871014 (Basic)
EP 240990 A3 880518
EP 240990 B1 910710
APPLICATION (CC, No, Date): EP 87105134 870407;
PRIORITY (CC, No, Date): US 849046 860407
DESIGNATED STATES: DE; FR; GB
INTERNATIONAL PATENT CLASS: **A61N-005/10**
ABSTRACT WORD COUNT: 276

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	EPBBF1	1342
CLAIMS B	(German)	EPBBF1	1213
CLAIMS B	(French)	EPBBF1	1543
SPEC B	(English)	EPBBF1	6733
Total word count - document A			0
Total word count - document B			10831
Total word count - documents A + B			10831

Method **and apparatus for selective irradiation of** biological materials.
Methode **et appareil pour irradiation selective de materiaux biologiques.**
INTERNATIONAL PATENT CLASS: **A61N-005/10**

...ABSTRACT A2
The invention relates to a **method** and an apparatus for frequency selective irradiation of biological materials and particularly tissues, cells and...

...treatment modality. Moreover, the source frequency can be adjusted to enhance the killing effect. The **method** and apparatus according to the present invention are useful in combination with naturally occurring or ...

...pharmaceutical stable isotope absorbers, having significantly reduced side effects as compared with conventional chemotherapy or **radiation therapy** .
The **method** and apparatus according to the invention can be applied for selective **radiation therapy** providing selective tissue damage or **necrosis** , in particular for **cancer** therapy, and for differentiating between diseased and normal tissues.

...SPECIFICATION B1

The present invention relates to a method and an apparatus for the selective, specific irradiation of biological materials and particularly of tissues and cells of living organisms, in particular of human and animals, and of cell components, with radiation of selective frequency.

Besides from non- therapeutical applications the method and apparatus according to the invention are optimally suited also for frequency selective radiation therapy for cancer .

In the treatment of tumors by ionizing radiation , x - rays or gamma rays are particularly used. The ideal in radiation therapy of malignant disease is achieved when the tumor is completely eradicated and the surrounding normal tissue, in the treated volume, shows little or no evidence of structural or functional injury. The important factor in successful treatment is the difference in radiosensitivity of neoplastic and normal cells. All tissues, normal and neoplastic , are affected by radiation so that radiosensitivity is a relative term. The basis of radiation therapy is that cells that are actively proliferating or that are of a primitive type are more sensitive than normal tissue so that there is usually a considerable margin between doses that are damaging to neoplastic and to normal cells. The difference depends on the capacity for intracellular repair of normal and neoplastic cells and the ability of normal organs to continue to function well if they are only segmentally damaged. If surrounding tissue can tolerate twice the radiation dose of a given tumor , then the tumor is radiosensitive.

Mammalian cells are capable of accumulating radiation damage before they are killed exponentially. Also, if allowed sufficient time after exposure, mammalian cells are capable repairing sublethal and potentially lethal radiation damage. The effects of x-rays or...

...of present radiotherapy is the DNA molecule of a cell which does not select for cancer cells but selects for DNA repair capabilities. Even a two-to-one increase in radiation sensitivity in cancer cells will result in a curable condition. However, normal surrounding tissue may not be more tolerant to x - ray therapy than cancer tissue which makes this therapeutic modality useless .

Mosbauer absorption , which is the resonant absorption of gamma rays by nuclei, represents a method of increasing the radiosensitivity of tumors in terms of orders of magnitude via selective energy deposition in cancer cells. Mosbauer radiation is completely analogous to optical absorption . In the latter, the ultimate source of radiation consists of excited atoms or molecules which...

...analogously to the dispersion device in optical absorption. By varying the driving frequency, a resonance system can be driven by the emitted gamma photons and the nuclear energy transitions of the sample (absorber). The absorber may occur naturally, or as in...

...embodiment, comprise added stable pharmaceutical isotopes, discussed below.

Furthermore, since it has been determined that cancer cells differ from normal cells with respect to level of aerobic versus anaerobic metabolism(sub(,)) internal concentrations of ions such as Ca(sup 2)(sup ...

...potentials, it is believed that such differences would cause differences in the nuclear microenvironment in cancer cells versus normal cells significant enough to result in excitation energy differences on the order of 10(sup -)(sup 6) eV. Such excitation differences will affect

Mosbauer absorption, and would allow for selective targeting of **cancer** cells. Thus, exposing **malignant** tissue with, for example, **an** (sup 5)(sup **7**)Fe absorber pharmaceutical to a narrow line width beam of 14,4 keV photons having...

...represents a powerful treatment modality.

It is an object of the invention to provide a **method** and an apparatus for the selective irradiation of **biological** materials and particularly of tissues, cells and/or cell components, and particularly for externally and...

...is accomplished by claims 1, and 23. The dependent claims refer to preferred embodiments.

The **method** according to the invention for the selective irradiation of biological target materials is **defined** in claim 1.

The apparatus according to the invention for carrying out the above **method** comprises a Mosbauer isotope (gamma)-radiation source (50) selectively emitting (gamma)-radiation,

filter means for...to the drawings, wherein:

Fig. 1 shows an apparatus for explaining the present invention,

and

Fig . 2 show an embodiment of an apparatus of the present invention.

The most dramatic killing...

...or is transmitted to another electron which is then ejected as an Auger electron. The **process** continues, shell by shell, until the valence shell is reached and thus leads to multiple...

...the s electron density, electric field gradient, and effective magnetic field at the position of **the** nucleus in which resonant gamma ray absorption occurs. Therefore, absorption is affected by the bonding...

...gamma rays resonantly absorbed, C.M., Makak, R.A., and Collins, R.L., Nuclear Instrument **Methods** , 114 (1974) 1. Approximately half of these electrons are emitted in the backward direction, which...

...not the case with particle radiation. The remainder of excited nuclei re-emit gamma or **x - rays** . Thus, Mosbauer **cancer therapy** promises the advantages of selective radionuclides without systemic radiation of normal tissue, higher kill per...

...by a term Eo(v/c) cos (sigma), where v is the velocity of the **atom** , **and** (sigma) is **the angle** between v and the momentum vector of the photon. The energy E(sub(v)) of...

...2) + Eo(v(')/c) cos(sigma)(') (2)

The energy of gamma rays emitted by a **system** of free atoms moving with thermal velocities would be centered at Eo - Eo/2m c...

...9)(sup 1)Ir nuclei in a solid do not obey Equation 1; instead, they **had** energy equal to Eo and a line width (see image in original document) where Tm...averaged over the lifetime of the nuclear excited state; (lambda) is the wavelength of the **radiation** . It can be **seen** from Equation 3 that f is large when the scattering center is confined to a...

...conversion coefficient (a describes the relative strength of radiative ((gamma)-ray) and nonradiative (electron conversion) **processes** connecting the ground and excited states; a= 0 if all the decays from the

excited...

...involve the emission of a (gamma)-ray). Thus the cross-section for the electron conversion **process** is a-times the radiative cross-section. The fact that this cross-section is dependent...

...:nucleus and is also very large compared to the photoelectric (electronic) absorption cross-section for **iron** , which is 5.5 10(sup -)(sup 2)(sup 1) cm(sup 2) per atom at this energy. The absorption is an exponential function of the cross-section; thus **the** nuclear resonance absorption is a strong effect.

The Apparatus

The overall operation of the **system** may be exemplified by the (sup 5)(sup 7)Co/(sup 5)(sup 7)Fe...

...energy.

By mounting the source on an accurately controlled mass drive, the energy of the **photon** is shifted by means of the Doppler effect. A velocity of 1 mm/s corresponds...radiation during the nonresonant excursion.

The source, or emitter of radiation, can also include the **techniques** known to Mosbauer spectrometry, with the addition of a single frequency filter 80. The radiation...

...the irradiated materials, particularly tissue components excited at the Mosbauer frequency can also be observed **from** the target area. The dynamic range (signal-to-noise) can be enhanced by viewing the...

...the treatment effectiveness. A control signal can be derived from such fluorescence, and obtained or **processed** by **processor** 94 according to the characteristic plot to continuously control the source to optimize the therapeutical...

...energy gamma photon, and most are stable isotopes; therefore, they can be used in scintiscan **methods** . As in the case of radionuclides, information can be gained based on differential uptake, excretion, or concentration as a consequence of the physiology of the pathological **process** . However, Mosbauer scintiscans also provide the ability to diagnose disease **processes** and to selectively image different tissues based on the phenomenon of the differential resonance frequency...

...the absorber isotope or isotopes by causing a selected Doppler shifted emission from the emitter **or** emitters **along** one axis and simultaneously scanning with conventional scintiscan instrumentation along an axis different from the...

...to attenuation of the exciting beam as a function of distance along the emitting axis, **a** correction algorithm has to be used to **process** the data to produce an image of the actual distribution of the Mosbauer isotope or isotopes in the **tissue** .

Radionuclides, which have short half lives, on the order of **hours** , and which are gamma-emitting isotopes, are used in scintiscans to gain diagnostic information based on the physiological properties of the pathological **process** . These properties include differential uptake, concentration, or excretion of the radionuclide by normal versus diseased ...

...on uptake of labelled colloid by Kupper cells, where (sup 1)(sup 9)(sup 8) **Au** colloidal gold or (sup(99m)Tc sulfur colloid is most commonly used; the HIDA or...

...and the gallium scan, in which the radionuclide (sup 6)(sup 7)Ga is concentrated **in neoplastic** or inflammatory cells to a greater degree than in hepatocytes. Hence, a hepatoma or liver...
...or "hot spot" with a gallium scan. The gallium scan is also helpful in diagnosing **neoplastic** infiltration in the patient with cirrhosis, since the **tumor** will show increased uptake, while fibrous bands will show decreased uptake. Another major application of...

...cystic duct or common bile duct obstruction. The normal physiology involved is the uptake of **these** compounds by the hepatocytes followed by excretion into the biliary canaliculi and concentration in the...

...absorption frequency in tissues. The stable isotope (sup 5)(sup 7)Fe demonstrates this effect; **thus** , cytochrome c which contains Fe can be selected **as** a target for Mosbauer absorption. Cytochrome c is a heme protein found in the mitochrondria...is significantly different from that of oxyhemoglobin. This property may be used to treat large **tumors** which have outgrown their blood supply and are therefore ischemic. By irradiating at the deox...

...gamma rays would be selectively absorbed by red blood cells present in vessels supplying the **tumor** . Coagulation secondary to damage to those cells would result in thrombosis of the blood supply to the **tumor** and concomitant **tumor** death.
(sup 5)(sup 7)Fe occurs with a natural abundance of 2,2%. Furthermore ...

...sup 7)Fe would incorporate this isotope in cells which have a rapid turnover rate. **Cancer** cells would be enriched relative **to** normal cells.
Many other stable isotopes demonstrate recoilless absorption of gamma ray photons following recoilless...

...decaying isotope. The absorber isotopes appear in Table 1. As exemplified by iron, these isotopes **may** be substituted into natural biological molecules or may be incorporated into a target tissue as non-naturally **occuring** pharmaceutical molecules. (**see** image in original document) (see image in original document) (see image in original document) (see...

...1)(sup 2)(sup 7)I and (sup 1)(sup 2)(sup 9)I could **be** used in hormones or (sup 6)(sup 7)Zn in enzymes. Also, Mosbauer isotopes could...

...a Mosbauer atom or molecule incorporating one or more Mosbauer atoms to selected sites in **tumor** cells, for example. Large local concentrations could be achieved through this **process** . (sup 5)(sup 7)Fe bleomycin, for example, has an association constant for DNA of...
...1)(sup 1)(sup 9)Sn(sup 2)(sup +). (sup 1)(sup 3)(sup 1) **Xe** or (sup 1)(sup 2)(sup 9)Xe which are membrane- **soluble** could be used to localize into the nuclear, mitochondrial or cellular membrane. Furthermore, experiments have...

...547; Giberman, E., et al., J Phys. 35 (1974) C6-371).
Tissue selective therapy
Bone **tumors** and bone metastases can be treated by the incorporation of a Mosbauer absorber into bone...

...Marshall , J.H., Phys Med. Biol. 13 (1968) 15).
In addition to the alkaline earths, **the** rare earths are also "bone

seekers." In vivo and in vitro fixation of rare earths...the metabolic incorporation at sites of new bone formation secondary to metastatic or primary bone **cancer** .

Also, pharmaceuticals, active ingredients and corresponding compositions could be synthesized using these isotopes such that the Mosbauer absorption occurs at a Doppler frequency in the **cancer** cells which is different from that of normal cells. The difference in chemical environments between normal and **cancer** cells results in alternate conformation, protonation, charge, etc. of the properly constructed therapeutic molecule so...

...s electron density results in a difference in the nuclear transition energy with a concomitant **frequency** difference of absorbed photons. Energy/frequency selective therapy

The Mosbauer absorption spectrum of a biopsy of normal and **malignant** tissue would yield the Doppler shifted **frequencies** that would result in selective gamma ray absorption in the **malignant** tissue. The apparatus and **methods** according **to** the present invention also select the source frequency to optimize the **cell** damage or **kill** when different from the actual Mosbauer absorption of the target tissue.

The photoelectric and Compton...

...in the absence of the Mosbauer effects. The equation for determining the total dose from **gamma** ray **treatment** and **the** depth of penetration of the photons is equation (11). Equation (11) and Table 2 demonstrate the relationship **that** photons of higher energy **penetrate** deeper into tissue. Since the different Mosbauer isotopes demonstrate a wide range of **photon** energies, therapies **can** be designed to exploit this phenomenon to deliver the energy of the radiation to a...

...7)Fe with a mass energy tissue absorption coefficient of 1,32 cm(sup 2)/**g** would **be** suitable for intraoperative **radiation** of breast, bowel and pancreatic **cancer** , whereas the 60 keV gamma ray of (sup 1)(sup 5)(sup 5)Gd with...

...sup 2)/g represents a suitable isotope for the treatment of primary and metastatic bone **cancer** . (see image in original document) (see image in original document)

The fluorescence absorption cross section...

...is given by: (see image in original document) where h is Planck's constant, c **is** the velocity of light, Eo is the transition energy, Ie and Ig are the excited...

...conversion coefficient. a is the ratio of the intensity of the internal conversion and fluroescence **processes** connecting the ground and excited states. This cross section is dependent entirely on nuclear parameters. The Auger **process** is the phenomenon useful in **cancer** therapy, and the Mosbauer nuclear cross sections of absorption followed by internal conversion of some...

...by: (see image in original document) where E is the incident (gamma)-ray energy and (**GAMMA**) is the uncertainty-principle energy width of the excited state. This width is defined by (GAMMA) =h/ **2** (pi)t , where t is **the** mean life (= 1,44 x the half life) of the excited state. The values of...

...susceptible to double strand breaks and one event which produces a double strand break could **kill** a **cell** . Conventional **radiation** **therapy** relies on nonspecific ionization and **free** **radical** production where H(sub 2)O is the primary target. If the total dose is...as 4

10(sup -)(sup 9) cm(sup 3), and the following equation was used: (see image in original document)

To calculate the reverse value, that is the effective radiation dose for Mosbauer cancer therapy, the following values are used: number of events necessary to kill a cell : 1; drug concentration: 10 (mu)M; volume of a cell nucleus: 2 10(sup 1...

...10(sup -)(sup 5))(2 10(sup -)(sup 1)(sup 2))(6 10(sup 2)(sup 3))(716 10(sup -)(sup 2)(sup 0))

= 3.5. 10(sup 1)(sup 0) photons which is approximately 3.5 rad.

If the depth of penetration desired is...

...dose of 350 rad is only 5,8% of the necessary effective dose by conventional methods , and increasing the concentration of the pharmaceutical containing a Mosbauer isotope will directly decrease this ratio. As a consequence of the low dosage of radiation required to be tumoricidal the source can be miniaturized and incorporated into instruments such as laparoscopes and bronchoscopes to...

...Auger cross-section and a high recoil free fraction which could be used for Mosbauer cancer therapy. Several of the isotopes are radioactive, but the half lives of these elements are so long that at the low concentration necessary for therapeutic effectiveness, not even the dose of a radioisotope scimtiscan which is about 1m Ci , is exceeded.

Example for (sup 4)(sup 0)K: t(sub 2) = 1,29 10(sup 9) a. (see image in original document) (see image in original document)

Furthermore, selective killing of cancer cells with sparing of normal cells can be achieved by several mechanisms:

1. The use of pharmaceuticals which are selectively taken up by cancer cells.

2. The use of pharmaceuticals which have a different isomer shift, quadrupole hf splitting or magnetic hyperfine splitting in cancer cells as compared with normal cells.

3. Applying magnetic or quadrupole fields in the space occupied by the cancer tissue so that a hyperfine absorption line is created for the cancer tissue which is absent for the normal tissue.

4. Polarization of the incident gamma rays with resonant polarization of the absorbers in the cancer tissue and not in the normal tissue.

In the latter case, polarized gamma rays can be obtained by three methods , magnetized ferromagnetic sources, quadrupole split sources and filter techniques , as shown by U. Gonser and H . Fischer, Current Topics in Physics, Mosbauer Spectroscopy, The Exotic Side of the Method : Resonance (gamma)-Ray Polarimetry, 99- 135 .

For cases 3 and 4

The nuclear spin moment of Mosbauer isotopes becomes aligned in an imposed magnetic field. Also when a magnetic field exists at the nucleus the nuclear quantum levels are split into sublevels which gives rise to hyperfine interactions and multiple transition energies between the ground and excited states. The application of a magnetic field in a certain region of space thus creates a new transition for a Mosbauer isotope present in that volume of space.

By driving the mass drive of the source 50, as shown in Fig. 2, at the proper velocity to produce a...

...as follows: (see image in original document)

Alternative combinations of therapeutic treatments

The two major cancer therapies are radiation therapy and chemotherapy. The latter includes agents which can be broken down into

six major classes...

...Cisplatinum is an alkylating chemotherapeutic agent which becomes covalently bound to DNA. Irradiation at a **distinct resonance** frequency **of Mosbauer** nucleus, (sup 1)(sup 9)(sup 5)Pt localized in the **tumor** cells combines the effects of MIRAGE therapy with that of chemotherapy to synergistically enhance **tumor cell death** . Another such example is the hybrid intercalating pharmaceutical, (sup 5)(sup 7)Fe Bleomycin.

As an alternative to selective **kill** of target **cells** due to irradiation at a frequency which is resonant only for the isotope localized to...

...taken up by the target cells. This is facilitated by the relative nontoxicity of any **pharmaceutical** distributed in nonirradiated areas. Also, the target tissue is irradiated locally; therefore, the enhanced **differential uptake would** only be relative to other cell populations in the radiation field.

The **method** and apparatus according to the present invention are also useful **for** the treatment **of** diseases other than **cancer** . The basis of therapy rests on the selective destruction of one or more cell lines ...

...to be an effective means of reducing inflammation, effusion, and pain in patients with rheumatoid **arthritis** . By using Mosbauer therapy where the stable isotopes (sup 1)(sup 6)(sup 1)Dy or (sup 1)(sup 6)(sup 3)Dy are substituted for (sup 1)(sup 6)(sup 5)Dy...

...the target cells. The subsequently released Auger electrons would destroy the target cells. Thus, the **cell line** responsible for disease can be eliminated without internalization of the hybrid molecule which is necessary...

...CLAIMS B1

1. A **method** for the selective irradiation of biological target material comprising tissues, cells and cell components, for externally and selectively inducing damage thereof,
   with exception of **methods** for the therapeutical and surgical treatment of the human or animal **body** and diagnostic **methods** practised on the human or animal body,
   characterized by
   taking a **target** material having incorporated therein a Mosbauer absorber, adjusting the Mosbauer absorption frequency thereof exclusively or...

...by irradiation with (gamma)-radiation from a Mosbauer isotope as (gamma)-radiation source.

2. The **method** according to claim 1, characterized by filtering **the** selective (gamma)-radiation frequency of the (gamma)-radiation source to provide a single frequency (gamma)-radiation.
3. The **method** according to claims 1 and 2, characterized in that the radiation emitted by the excited Mosbauer absorber of the irradiated target material is detected.
4. The **method** of claim 3, characterized in that **the** frequency of the emitted radiation of the (gamma)-radiation source is adjusted according to the detected radiation of the excited Mosbauer absorber of the irradiated target **material** .
5. The **method** according to claim 3, characterized in that the fluorescence radiation emitted by the irradiated target material is detected in a direction different from the axis **direction** of the incident radiation.

6. The **method** according to one of claims 1 to 5, characterized in that the irradiated target material is imaged according to the detected radiation of the **excited** component.
7. The **method** according to claims 1 to 6, characterized in that an active ingredient, particularly a pharmaceutical or a pharmaceutically active ingredient, or a pharmaceutical composition is added to the **material** to be irradiated and/or administered to an organism, comprising a Mosbauer absorber to be excited selectively.
8. The **method** according to claim 7, characterized in that active **ingredients** , pharmaceuticals and/or compositions are used which consist of a Mosbauer absorber isotope or comprise a Mosbauer absorber isotope.
9. The **method** according to claims 7 and 8, characterized in that a **Mosbauer** absorber isotope is used which is localized at specific sites in the target material.
10. The **method** according to claims 8 and 9, characterized in that a Mosbauer absorber isotope is used which is selected according to its affinity for the target material.
11. The **method** according to claims 8 to 10, characterized in that the active ingredients, pharmaceuticals and/or compositions are administered selectively to the material to be irradiated according **to** the blood flow of the material to be irradiated, wherein the amount of said isotope applied corresponds to the blood concentration in the target material.
12. **The method** according to claims 8 to 11, characterized in that for providing an increased concentration of the radiation of the excited Mosbauer **absorber** active ingredients, pharmaceuticals and/or compositions comprising a Mosbauer isotope are used which is selected according to the differential isotope uptake of the target material.
13. The **method** according to claims 8 to 12, characterized in that active ingredients, pharmaceuticals and/or compositions comprising a Mosbauer isotope are used which is selected according to the effective **penetration** depth of the corresponding Mosbauer frequency of said isotope at the target material.
14. The **method** according to one of claims 8 to 13, characterized in that active ingredients, pharmaceuticals and/or compositions are used **wherein** the Mosbauer isotope is bound to a carrier substance having an affinity for the target material.
15. The **method** according to claim 14, characterized in that active ingredients, pharmaceuticals and/or compositions are used...

...carrier substance represents or comprises a monoclonal anitbody and/or a natural hormone.

16. The **method** according to claims 8 to 15, characterized in that active ingredients, pharmaceuticals and/or compositions **are** used which represent or comprise at least one of the following nuclides as Mosbauer absorbers...

...in original document) (see image in original document) (see image in original document)

17. The **method** according to claims 1 to 16, characterized in that at least one of **the** following nuclides or at least one of the following nuclide combinations, respectively, are used as...

...in original document) (see image in original document) (see image in original document)

18. The **method** according to claims 1 to 17, **characterized** in that Mosbauer absorbers and Mosbauer sources are used which are selected from the following...

...see image in original document) (see image in original document) (see

image in original document)

19 . The **method** according to claims 1 to 18, characterized in that components for selective absorption of the...

...radiation emitted from the (gamma)-radiation source are used which represent or comprise a molecule, **particularly** a protein molecule and/or a peptide molecule.

20. The **method** according to claim 19, characterized in that the components for selective absorption of the (gamma)-radiation include naturally occurring and/or synthesized elements.

21. The **method according** to claims 1 to 20, characterized in that the irradiation is carried out such that tissue target material undergoes **necrosis** .

22. The **method** according to claims 1 to 21, characterized in that the target material is provided with (sup 5)(sup 7)Fe-Bleomycin.

23. An apparatus for carrying out the **method** according to claims **1** to 22, comprising a Mosbauer isotope (gamma)-radiation source (50) selectively emitting (gamma)-radiation, filter for location in the target **material** to be irradiated.

24. The apparatus according to claim 23, characterized by a Doppler shift ...

...23 and 24, characterized in that the filter means (80) comprise crystal diffraction means (84).

26 . The apparatus according to claims 23 to 25, characterized by means (92) for detecting the...

...to 26, characterized in that the Mosbauer absorber being the component to be excited of **the** target material is designed or selected such that it emits a radiation of sufficient intensity to cause damaging or **necrosis** of the irradiated target material.

28. The **apparatus** according to claims 23 to 27, characterized by means (92) for detecting the fluorescence radiation from the target material caused by the irradiated filtered (gamma)-radiation in a direction different **from** the irradiation direction of the (gamma)-radiation.

29. The apparatus according to claims 23 to 28, characterized by means for imaging the **irradiated** target material **according** to the radiation emitted therefrom.

30. The apparatus according to claims 23 to 29, characterized...

...comprised in a molecule, particularly a hormone, a protein molecule and/or a peptide molecule.

31 . The apparatus according to claim 30, characterized in that the Mosbauer absorber includes or consists

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 240 990**
A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **87105134.8**

(51) Int. Cl.⁴: **A61N 5/10**

(22) Date of filing: **07.04.87**

(30) Priority: **07.04.86 US 849046**

(43) Date of publication of application:
14.10.87 Bulletin 87/42

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(71) Applicant: **Mills, Randell L.**
R.D. 2
Cochranville Pennsylvania 19330(US)

(72) Inventor: **Mills, Randell L.**
R.D. 2
Cochranville Pennsylvania 19330(US)

(74) Representative: **Patentanwälte Beetz sen. - Beetz jun. Timpe - Siegfried - Schmitt-Fumian**
Steinsdorfstrasse 10
D-8000 München 22(DE)

(54) Method and apparatus for selective irradiation of biological materials.

(57) The invention relates to a method and an apparatus for frequency selective irradiation of biological materials and particularly tissues, cells and/or cell components of living organisms, in particular of human and animals, by irradiating a component element of the target material at the corresponding Mößbauer absorption frequency. The component radiation absorption at the Mößbauer absorption frequency is thus enhanced many times over the absorption of the surrounding material having a different Mößbauer absorption frequency. The energy thusly absorbed by the target material component is converted to and remitted as Auger electrons, which provide intranuclear radiation resulting in lethal double strand breaks in the DNA molecules of the target. The irradiation is carried out in frequency and material selective modes, and may be combined with conventional chemotherapeutic agents to provide a further enhanced treatment modality. Moreover, the source frequency can be adjusted to enhance the killing effect. The method and apparatus according to the present invention are useful in combination with naturally occurring or administered pharmaceutical stable isotope absorbers, having significantly reduced side effects as compared with conventional chemotherapy or radiation therapy.

The method and apparatus according to the invention can be applied for selective radiation therapy providing selective tissue damage or necrosis, in particular for cancer therapy, and for differentiating between diseased and normal tissues.

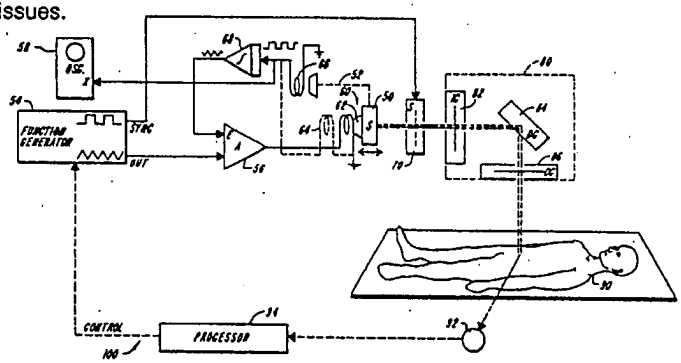


FIG. 1

EP 0 240 990 A2

25/3,K/8 (Item 8 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00152243 **Image available**
APPARATUS PROVIDING DIAGNOSIS AND SELECTIVE TISSUE NECROSIS
APPAREIL PERMETTANT LE DIAGNOSTIC ET LA NECROSE SELECTIVE DE TISSUS
Patent Applicant/Assignee:
MILLS Randell L,
Inventor(s):
MILLS Randell L,
Patent and Priority Information (Country, Number, Date):
Patent: WO 8809152 A1 19881201
Application: WO 88US1796 19880527 (PCT/WO US8801796)
Priority Application: US 87591 19870528
Designated States: AT AU BE BR CH DE FR GB IT JP KR LU NL SE SU
Publication Language: English
Fulltext Word Count: 67628

APPARATUS PROVIDING DIAGNOSIS AND SELECTIVE TISSUE NECROSIS
Main International Patent Class: **A61B-019/00**
Fulltext Availability:
Detailed Description
Claims
English Abstract
Pharmaceuticals and apparatus used in combination for diagnosis and tissue **necrosis** applicable to provide effective and selective therapy using the Mossbauer absorption phenomenon. Selected pharmaceutical compounds...

Detailed Description
APOARATUS PROVIDING DIAGNOSIS
AND SELLECrIVE TISSUE **NECROSIS**
CROSS REFERENCE TO RELATED APPLICATIONS
This application is a continuation-in-part of my co...

...Mossbauer effect for
diagnostic and therapeutic purposes.

BACKGROUND OF THE INVENTION
In the treatment of **tumors** by ionizing radiation, typically X-rays or gamma rays are used.

The ideal in **radiation therapy** of **malignant** disease is achieved when the **tumor** is completely eradicated, and the surrounding normal tissueo in the treated volume, shows little or...

...or
functional injury. The important factor in successful treatment is. the difference in radiosensitivity of **neoplastic** and normal cells, All tissues, normal and **neoplastic** , are affected by radiation so that radiosensitivity is a relative term, The basic consideration of **radiation therapy** is that cells that are actively :proliferating or that cells which are of a primitive...

...tissue so that there is usually

a considerable margin between doses that are damaging to **neoplastic** and to normal cells. If this is the case, then a multifraction dose schedule decreases the size of the **tumor** over time while permitting time
SUBSTITUTE SHEIET
between doses for normal tissue to recover, A constant fraction of **tumor** **cells** are **killed** with each treatment, and theoretically the **tumor** can be completely eliminated with a sufficient number of treatments. However, normal tissue has a...

...s history is eventually reached. Exceeding this threshold results in unacceptable side effects. @Thus, the **tumor** volume must be reduced sufficiently before the threshold is reached or the **cancer** is incurable by this modality of therapy.

SUMMARY OF THE INVENTION
The present invention is pharmaceuticals,.

.apparatus, and a **process** which provides diagnosis, therapy and other biological effects by use of highly selective absorption of...analogously to the dispersion device in optical absorption. By varying the.driving velocity, a resonance **system** can be driven by the emitted gamma photons with regard to the nuclear energy transitions...

...biological target such as the DNA of the target tissue as part of a therapeutic **process** , Alternatively, the present invention provides diagrams by monitoring the release of nonlethal energy,, as described...polarization and propagation direction conditions to achieve resonance in the MIRAGE absorber pharmaceutical. The- Apparatus, **Systems** , Compounds, **methods** ., and specifications; of use are described in detail below,
BRIEF DESCRIPTION OF THE DRAWINGS
These...

...detailed description taken together with the drawing, wherein.

Figo 1 is one embodiment of the: **system** apparatus of the present invention;
Fige 2 is an alternate embodiment of the **system** apparatus@ of the present invention;
Fige 3 is an alternate embodiment of a portion of the **system** of; Figs., I or 2., showing the position Of surface coils;
Fige 3A is@ a...

...isometric view of an alternate embodiment of an array of coils for use in the **system** apparatus of Figs. 1 and 2;
S U - 2 S T! T U " '-!"E SLI
Fig. 7 is an isometric drawing of a **system** according to the present invention showing ultrasound

modulation of the gamma ray source and the...at the target area;
Figs. 8 and 9 are graphical plots of data
related to **radiation therapy** ;
Figs, 10A, B and C are diagrammatic
representations of the MIRAGE pharmaceutical 12/29/w...

...a 3/2@1/2 transition
in an oriented absorber with a unique principle axis
system ; and
Figs. 17A and B are the spectra from, a single
crystal of a.-Fe...

...Urn ST ITUTE. SIN EMT
DETAILED DESCRIPTION OF THE INVENTION
The present invention includes the **process** of
producing pharmaceuticals having desired Mossbauer
nuclear parameters such that they possess physical
5- and...
...the target tissue via the Mossbauer
effect,
The pharmaceuticals of the present invention and
the **process** of producing -the pharmaceuticals is
discussed first,, which. is followed by the apparatus
used in combination with selected pharmaceuticals to
effect the Mossbauer absorption in a biological
target as a **process** of the invention to provide a
therapeutic or diagnostic function. The -lattert
apparatus., provides a...the
pharmaceutical molecules present in the target
selected tissue.

SUSST.47UTE SHEET
Implementation of the **process** for making MIRAGE
pharmaceuticals involves selecting an atom responsive
to the Nossbauer effect at a...includes a polarizing element, to polarize
the
emission. Polarized gamma rays are obtained by three
methods : magnetized ferromagnetic sources,,
quadrapole split sources., or filter **techniques** , In
addition, the apparatus possesses means to produce
external magnetic fields and ultrasonic beams to...producing means are
described
below in the Apparatus Section,
ST jai
SHE-EET
- 15
The **process** of providing selectivity by
imparting magnetic fields with the apparatus involves
providing a magnetic field...

...of the nonselected tissue to the gamma
rays and absorption by the selected tissue.

The **process** of treatment involves using the
pharmaceuticals and apparatus in combination to cause
the Mossbauer ef...direction is applied,
or an ultrasonic beam i-s applied, For the ultrasonic
case, the **process** of effecting selectivity by causing
an ultrasonic beam to intersect the administered
gamma ray beam...

...by changing the direction of the source polarization magnetic field in the case of a **ferromagnetic** source), the magnetic **field** strength gradient (e.g, by changing the current in the surface coils which give rise...the source of fluorescence is used in a feedback loop which feeds into a control **system** which changes'the magnetic field strength and direction; ultrasonic beam frequency, direction and power; and...

...or past experience.

A representative calculation of an effective photon flux for treatment to achieve **necrosis** , and the associated dose appears in the Theoretical Section as does the theory of achieving selectivity by the modes mentioned, (Implicit is that the **process** for diagnosis is the same as that for treatment with regards to excitation, Detection is...Testing Of MIRAGE Treatment Using MIRAGE Drug 12/29/w
The human colon and breast **cancer** cell lines, HT29 and MCF7, respectively, were obtained from Cambridge Research Lab Inc.,, and were...
...Lab Inc.,, which the KundsIn Lab tested for these organisms. The human breast and lung ' **cancer** cell lines@,. HTB26 and A549, respectively, were obtained from the American Type Culture Collection, The...

...normalized to that of the control,
RESULTS
The effects of lm rad levels of Mossbauer **radiation** absorbed during MIRAGE **treatment** of the **cancer** cell lines-MCF7, McCoy, HT29., HTB26, and A549 using the MIRAGE drug 12/19/w...for the elimination of a pathological cell population, Previous experiments demonstrated that the most potent **killing** effect in **cells** by radiation is from secondary particles produced by internal conversion of gamma ray energy followed...

...nontoxic levels of radiation which are six orders of magnitude less than that of conventional **radiation** **therapy** where the Mossbauer effect was exploited for treatment, The ability to control the occurrence of...bronze. ;/i
Ne,014(cL
A
@b 0
'ary
SUSSTITUTE
SHE CET
(.Orqan.ometallic Compounds **Methods** of Synthesis Physical Constants and C@emical Reactions, Michael Dubb, Editor, 2nd Edition, Vol* III...with NaOH and copper bronze to yield the antimony derivatized acridine 19 according to the **method** of O'Donnell, G.J., Iowa State Coll, J.

Scior 20t 34-6 (1945); CA...dilithium naphthalene 39, is reacted with

tellurium to yield the product 40 according to the **method** of Marfat, A,, et al, Journ al of the American Chemical Society,, 220, (1977) ppe...53, is reacted with trimethyllithium germanide, 15,. to give the product 55, according to the **method** described in Comprehensive organs Metallic: Chemistry, Sir Geoffrey Williams, Editor (19Z2) Vol, 2,, Cho 10...8-hydroxyquinoline which intercalates DNA directly.

is synthesized using the indicated Mossbauer isotopes by the@ **procedures** referenced in The Actin de Elements, K*We Bagnall, (1972) pps 211-229, incorporated by...

...103 is prepared as follows.

Methylalcohol derivatized osmocene 101, which is prepared according to the **method** described in Comprehensive Organometallig ChemistjX, Geoffrey Wilkinson, Editor, (1982),q Vole 41 Pe 1018 (incorporated...compound 130, is reacted with iridium adduct 131 to give the product 132 by the **procedure** described by Gardner, S.A., et al,, Journal of Organometallic Chemistry, 60 (1973) 179-188...

...reacted with diazonium adduct 134 to give the o-metallated adduct 135 according to the **method** of Farrell,. N.; et al, Journal of the Chemical Society, Dalton, Trans., 1977, 2124j, incorporated...

...with Grignard reagent 139 followed by chlorination to give chloride adduct 140 according to the **procedure** of Rausch, M.D.

and Moser., G.A.,, Inorganic Chemistry, Vol. 13,, No. 1,, 1974...

...is reacted with phosphine compound 147 to give o-metallated adduct 148 according to the **procedure** described in Comprehensive Organometallic Chemistry., Geoffrey Wilkinson., Editor,, (1982)f Vole 5.* ppe 578-5870...and by isolating the product by filtration of evaporation of the solvent using reactions and **techniques** generally known to one skilled in the art.

...protecting vials and may be refrigerated if necessary,
THE APPARATUS
The overall operation of the **system** may be exemplified by the Co 57 /Fe 15 7Mossbauer pair as follows: the radioactive...changing the ultrasonic-.driving frequency.

The source, or emitter of radiation,, can also include' the **techniques** known to Mossbauer spectroscopy of narrowing the line width or absorbing unwanted Mossbauer lines, In...

...in Fig. 1 to
give a characteristic plot of the treatment
effectiveness. A spatially distributed **system** of.

multiple detectors such as proportional counters or
SUBSTMTESHEET
scintilation detectors, or lithium drifted silicon...of
treatment. A control signal can be derived from the
f luorescence, and combined or **processed** by
processor 94 of Fig. I according to the orientation
of detectors which record signal direction and...

...relative to the patient as shown in
Fig* 2 and transverse to the patient. A **system** of
such Helmholtz·coils are used as described below to
effect the field characterstics necessary...

...so that resonant absorption can be
localized to specific dimensions (such as that of a
tumor) while maintaining nonresonant,, and therefore
nonabsorptive, conditions in the surrounding
nonselected tissue at the energy...rays' propagation
direction, and selective absorption will occur for
the Am = 0 line by the **process** described in the
Theoretical Section.

An alteration of this scheme is to use two pairs...realized
selectivity by polarization and energy mechanisms
discussed in the Theoretical Section.

In a preferred **method** where f ields are used to
achieve selectivity, treatment is carried out so that
the...lenses and *collimated transducers to
produce; narrow directed-- ultrasonic beams are
described in Medical imaging **Systems** , Albert
Macovsik, ('1983), pp 173-223, incorporated by
reference.

Treatment is performed by directing the...common to both the path 212 of
the
gamma rays and the beam 206 of **acoustic** energy.

Treatment can be controlled by a microprocessor
which receives digitized input from peripheral
sensors which follow...

...SUMCN-f FTUTT SHEET
of magnitude greater than the processing times of
high speed Control **systems** .

ADDITIONAL APPLICATIONS
MIRAGE drugs and therapy have many diverse
applications in addition to the treatment of **cancer** .

For example, MIRAGE compounds can be used for imaging
and for treatment of any disorder...

...used in scintiscans to gain diagnostic
information based on the physiological properties of
the pathological **process** . These properties include

differential uptake, concentration, or excretion of the radionucleotide by normal versus diseased...

...by hepatocytes, and the gallium scan., in which the radionuclide 67 Ga is concentrated in **neoplastic** or inflammatory cells to a greater degree than in hepatocytes, Hence,, a SUBS if ruTrr...

...or "hot spot" with a gallium scan, The gallium scan is also helpful in diagnosing **neoplastic** infiltration in the patient with cirrhosis, since the **tumor** will show increased uptake, while fibrous bands will show decreased uptake. Another major application of...

...on differential uptake, - excretion, or concentration as a consequence of the physiology of the pathological **process** . But,. Mossbauer scintiscans also provide the ability to diagnose disease **processes** and to selectively image different tissues based on the phenomenon of the differential resonance frequency...

...function of distance along the source axis, a correction algorithm has to be used to **process** the data to produce an image of the actual distribution of the Mossbauer isotope or...

...AUTQIMMUNE, AND TRANSPLANTATION REJECTION DISEASE
A successful treatment for rheumatoid arthritis is@ the induction of **necrosis** of synovial cells of afflicted joints. For example, intra-articular radioactive synovectomy using the: radionucleotide...

...inflamation, effusion and pain in patients with rheumatoid arthritis.

- MIRAGE ther apy provides selective cellular **necrosis** and intra-articular MIRAGE synovectomy can be substituted for intra-articular radioactive synovectomy to give...

...by substituting stable Mossbauer absorber isotopes 1 6 5 for radioactive Dy in the synovectomy **treatment** ., systemic **radiation** exposure from leakage is avoided.

Ferric hydroxide macroaggregate is massive, in a recoil sense andOther diseases which can be cured by inducing **necrosis** of specific cell lines include autoimmune diseases and transplantation rejection disease which includes graft versus...

...such as carboxyl, amino, sulfide, halogen, or carbonyl and condensing the two entities together by **methods** generally known to one skilled in the art, The

protein binds to surface of the...

...and involved in
atherosclerosis
The occlusion of arteries is the end result of
the atherosclerotic **process** which involves t
following stages 1) repeated injury which denudes the
vessel of endothelium, 2...

...which make up the vessel
wall. This is possible., however, with MIRAGE drugs
which can **kill** **cells** which have: incorporated the drug
by using levels of radiation which pose no threat to...000 deaths per
year which compares
with 30,000 deaths per year due. to breast **cancer** ,
AIDS is a fatal disease with no specific treatment,
and development of a vaccine presents...

...from other human pathogenic viruses
because it destroys the T cell segment of the immune
system which normally is responsible for controlling
SUBSTMUTF nwrwr
the elimination of a viral challenge. In...

...cytopathic.
Also,, the biology of the virus is such that it can
elude the immune **system** during -a latent phase and
then activate to produce virus at a tremendous rate
before the host **cell** **dies** . This life cycle is a
consequence of a transactivating factor, tat III, and
trs, a...

...may require rapid viral protein synthesis and
assembly in the race between virion release and **cell** .
death . The presence of large amounts of tat III at
the time of a trs-mediated...can only slow the relentless progress of
this
disease which destroys the host's immune **system** by a
T cell cytopathic life cycle, The viral message
exists in the host DNA...

...in an
infected individual is to destroy all such cells
before the host's immune **system** is inundated with
virus and irreversibly compromised. MIRAGE drugs
represent agents which can selectively discriminate...
...environment at the Mossbauer atom of an
intercalating MIRAGE drug can be exploited cis a
method to selectively eliminate HIV infected cells in
the latent stage, Tat III is the only...

...drug followed by systemic irradiation at the
frequency of the created isomer shift will
selectively **kill** latent infected **cells** and interrupt
the infectious **process** ..

THEORETICAL SECTION
PRINCIPLES OF **RADIATION** **THERAPY**
Ionizing **radiation** was found shortly after its
discovery to be capable of reducing the growth of

human **tumors** . Unfortunately the limitations of this modality were -discovered as patients developed catastrophic late compiications@, The...

...must perform treatment such that the balance of these opposing ends is in favor of **tumor** ablation. The.

total story of the cellular mechanisms involved remains elusive; however,, many, of the...

...basic understanding of the effect of radiation on cells and the cellular response to damage.

Radiation therapy involves; particle and electromagnetic radiation which causes damage to both normal and **cancer** tissue. The goal is to ablate the **tumor** while preserving normal tissue. The principles involved are manifested in cell survival curves. In Fig...for ml and cc2 .
*9 where 3 doubling times. Cells
SUBS, -?UTE SHEET
exposed to **radiation** reach a **treatment** threshold and then are killed exponentially, the survival number versus radiation dose is an exponential curve where a constant fraction of the **cells** are **killed** per treatment. All **tumors** can be controlled as 'the dose goes to infinity; however, it is the limitation of tolerance of normal tissue not the ability to control the **tumor** . which is the guide to treatment, Thus,, it can be appreciated that a significant 'factor...
...first
order rate equation below.

cl. Dose
(6)
N
critical is a reduction of the **tumor** burden, N,, to a level which is no longer overwhelming to the body's natural defenses.

Treatment with **radiation** can lead to a cure even though this is a local modality which has no effect on distant micrometastases despite the shedding of **malignant** cells by **tumors** , which are below the mass sufficient for diagnosis. Current data supports three explanations for this...

...host has the ability
to kill a limited number of viable metastatic cells,
(3) The **tumor** mass influences its own metastatic potential. **Radiation therapy** by diminishing the mass reduces the source of clonogenic metastases and increases the host's ability to deal with
SUBOT"UM SHFET
residual micrometastases by eliminating the **tumor** 's adverse effect on the host immune **system** , The ideal in **radiation therapy** of **malignant** disease is achieved when the **tumor** is completely eradicated and the surrounding normal tissue of the

treatment volume is structurally and...

...intact The important factor in the successful treatment is the difference in the radiosensitivity of **neoplastic** and normal cells which is the slope,, M, of equation 6. The difference depends on...

...damaged, In general, if surrounding tissue can tolerate twice the radiation dose of a given **tumor** .. then the **tumor** is radiosensitive. Alternatelyt a **tumor** which extensively involves both lungs, and may be cured with a dose of 3000 rads...
...with radi ation therapy because of- the greater radiosensitivity of the' surrounding lung .tissue.

All **tumors** can be eradicated by t reatment with sufficient radiation, But'.. damage to normal tissue is dose limiting due to the acute and late ef f ects of **radiation therapy** , Acute effects ...total dose limiting in radiation. They often progress with time and are usually include fibrosis, **necrosis** , irreversible, These fistula formation, non-healing ulcerations, and damage to specific organs such as spinal...

...misleading as a guide to long term effects, There are a number of examples in **radiation therapy** where the total dose has been increased,, the size of the dose fraction increased or...

...depletion of the stem cell pool. Acute ef f ects depend on the balance between **cell killing** and compensatory replication of both, the stem and proliferative compartments, The development of late effects...

...that the stem cells have only a limited proliferative capacity. Compensation for extensive or repeated **cell death** may. exhaust this capacity. resulting in eventual organ failure. This phenomenon can be demonstrated for...

...into irradiated mice until they lose the abillity to reconstitute the: recipient-'s marrow,
Successful **radiation - therapy** can be understood from the dynamics of cellular responses to radiation. From the dynamic point of view, the basic difference between a normal renewal tissue of the body and a **tumor** is that in normal tissue there is an effective balance between cell production and cell loss; whereas@, in **tumors** ,, cell proliferation exceeds cell loss, The normal renewal tissue can be considered a ...of three types of cells: Stem cells -+ Maturing cells -# Functioning cells.

The cell cycle of **cancer** cells are- in general shorter than those of normal tissue. It is f ound in general that irradiation causes an elongation of the generation cycle of **tumor** cells while a corresponding shortening of the cell cycle of normal cells is the norm...

...is
enhanced if post radiation conditions are suboptimal for growth. Both of these mechanisms· favor **tumor**· cells over normal cells.

Thus, a major factor leading to a cure and which underlies...

...dose regimens
that are so commonly employed in clinical radiotherapy.
As with normal tissue, different **tumors** have a range of radiosensitivity some being responsive to a few hundred rads, and others...

...as much
as 10,000 rads, and this variation can even exist within a specific **tumor** type. Furthermore, radioresistance is selected for in the **tumor** population as normal tissue regenerative capability declines, Thus., 'it can be appreciated,, from survival curves...

...in Figs. 8 and 9,, that
necessary but not sufficient conditions for a cure via **radiation therapy** are that the first order kinetics of **cell kill** must be such that enough **cancer cells** are **killed** and the **tumor** does not return to its original mass in the time interval necessary for normal tissue to regenerate. And, the **tumor** volume is reduced to a level which can be eliminated by the host's defenses...
...will ultimately produce unacceptable late effects.

SUES I I I U I SHEET
PHYSICS OF **RADIATION THERAPY**
Ionizing **radiation** exerts its effects on atoms primarily as a function of the number of electrons .

Biological...but
lacks the ability to repair double strand breaks which is the lethal event in **radiation therapy** .

The **radiation** effects ' on particular molecules such as DNA, are ascribed to two **processes** , direct and indirect action,- By direct action is meant the effects of energy directly in...probability as demonstrated by the inverse
relationship between the number. of decay events needed to **kill** a given **cell** type bya radioisotope and the number of radiated electrons which it produces.

SUIssTITUTE SHEET
PCT...

...electron which is then
ejected as an Auger electron to produce a new
vacancy, The **process** continues shell by shell, until
the valance shell is reached and thus leads to
multiple...

...an Auger cascade
which cause radiolysis and double strand breakage is
lethal to a cell, **Radiation therapy** is far less
efficient requiring approximately 105 photons
absorption events per cell to produce the with electromagnetic radiation
doses one million times less than that of
conventional **radiation therapy** , This is. accomplished
by utilizing phenomenon common to electromagnetic
radiation therapy and radioactive atomic DNA
labeling, MIRAGE therapy entails using Mossbauer
atomic labeled pharmaceuticals which bind...

...the same as@ for the case of 125,
labeled DNA.' Furthermore, this single: event will
kill the target **cell** which is in contrast to
conventional **radiation therapy** where mu-ltiple
improbable events must occur simultaneously to
produce a double strand break. 105...

...needed for MIRAGE. therapy.

The absorption cross-section for water the primary
target of conventional **radiation therapy** is
approximately 10 -25 CM2 whereas the resonant
17
cross-section for Mossbauer absorption , is 10
25 cm which represents an eight order of magnitude
improvement. This increased efficiency permits **cell**
kill with radiation doses of one millionth that of
conventional therapy.

PHYSICS AND-CHEMISTRY OF MIRAGE...pharmaceutical
molecule that permits the use of this phenomenon to
selectively treat disease such as **cancer** .

The Mossbauer effect is degraded by recoil
energy of the emitted and absorbed photon. This...

...the recoilless or recoil-free
fraction. To increase the relative strength of the
recoilless resonant **process** , it is important that f
be as large as possible. The recoilless fraction f
can...molecule.

As described previously, Auger cascades in DNA
binding pharmaceuticals cause DNA radiolysis -and
concomitant **death** of the **cells** in the target tissue.

The equation which relates the number of internal
conversion events with...

...respectively,
SUISSTMOE SHEET
alpha Table 8
B= beta Representative Mossbauer Isotopes with Parameters Favorable for **Cancer** Theral
Half Life Gamma Half Life /Auger Mossbauer
of Ground Isotope Ray of Excited (Cross...levels and well below levels that are necessary to
cause acute or late effects of **radiation** **therapy** ,
Furthermore,,, MIRAGE **therapy** is a modality whereby the side effects of chemotherapy can be eliminated.

MIRAGE drugs are...

...Te and other isotopes from
Table 7 appear in the Exemplary Material Section.

SELECTIVITY
Selective **killing** of selected' **cells** with sparing of nonselected cells can be achieved by several mechanisms.

SUBS I I rUTE...the side bands,
For case 1
MIRAGE therapy can achieve selectivity in the case of **cancer** therapy in animals including humans via exploiting known selective uptake by **cancer** cells of compounds such as Bleomycin,, cationic lipophilic dyes such as Rhodanine, hematoporphryins, and monoclonal...

...bound to the
compound known to be selectively taken up by the
SUBOTTrUTE SHEET
- 162
cancer , In contrast to chemotherapy., the selectivity need only be relative to other cell types in...

...such as
carboxyl, amino, sulfide, halogen, or carbonyl and condensing the two entities together by **methods** generally known to one skilled in the art.

Colloids such as those of gallium are known to be concentrated by certain types of **cancer** cells and the same phenomenon is predicted for certain colloids of Mossbauer isotopes comprising massive...

...incorporated into biological matrices including bone which is useful for the treatment of metastatic bone **cancer** . Examples include 40 Kr 15 3 Gdv
3G 161 Dyr 1 '3 Yq: 14gMt lsiuj...

...the precursor molecules of
thyroid hormones. All can serve as targets for treatment of thyroid **cancer** with MIRAGE therapy, And 57 Fe can be incorporated into heme proteins and red blood...frequency of deoxyhemoglobin which differs from that of oxyhemoglobin to exploit the relative hypoxia of **tumors** where hypoxia result' in a greater

concentration of deoxyhemoglobin. Furthermore, damage to the red blood cells in the **tumor** leads to coagulation followed by thrombosis of the blood supply to the **tumor** and concomitant **tumor** death.

For case 2
The energies of the nuclear states are weakly influenced by the...result of the presence of an internal magnetic field which can be generated by an **unpaired electron** in the **atomic** environment that can induce an imbalance in electron spin density at the nucleus or by...realized in the selected cells which is different from that of nonselected cells, For example, **cancer** cells are known to have differences in ion concentrations and ph from normal cells. Binding...only for the: proper spin moment alignment, Polarized gamma rays can be obtained by three **methods** ,, magnetized ferromagnetic sources, quadrapole split sources,, or filter **techniques** as shown by U. Gonser and H. Fischer, Current Topics in the Physics of Mossbauer Spectroscoloy, The Exotic Side of the - **method** .

Resonance Gamma Ray Polarimetry, 99 1 incorporated by reference,
Selectivity via polarization of the source...

...radiation and its dependence on orientation are determined by conservation of angular momentum in the **system** . of nucleus plus gamma ray (quantum selection rules) where the quantum-mechanical **treatment** of electromagnetic **radiation** leads to the introduction of photons which are bosons. of vanishing rest mass and quantized...the Am = 0 lines become strong.
Selective eradication of a selected cell line such as **cancer** tissue can be achieved by polarizing the **cancer** tissue with an orientation different from surrounding normal tissue and by irradiating with radiation which...

...referring to Figs. 17A and B, the nuclei of the MIRAGE pharmaceutical present in the **cancer** tissue can be aligned perpendicularly to the propagation direction of the gamma ray; whereas, the...

...By irradiation with gamma rays@ which are resonant with the Am = 0 transition,, only the **cancer** tissue will absorb the radiation.

SUBS I I rjjTE SHEET
- 175
For Case 5
The...in the absence of the Mossbauer effects, The equation for determining the total dose from **gamma** ray **treatment** and the depth of penetration of the photons appears@ in Table 11.

SUBSTMUTE SHEET
PCT...

...radiation using a miniturized source and mass drive or ultrasonic drive. Breast, bowel, and pancreatic **cancer** are candidates for the former; and lung **cancer** is a candidate for the latter, Mossbauer sources of high energy gamma rays which penetrate deeply can be used- to treat **tumors** that are not located superficially. 155 Gd is the source of a 60 KeV Mossbauer...

...gm and represents a sui@Eable source for the treatment of primary and metastatic bone **cancer** and deep solid **tumors** ,
SUBSTMUTE SHEET
i33Hs 3.Ln.Lusens
00 0% tA w tQ
in b zz im...TELLURIUM-125 DAUGHTER IS STABLE*
SUBSTITUTE SHEET
Modifications and substitutions of the compounds, pharmaceuticals, apparatus, **methods** , **systems** , and **process** , steps, made by one skilled in the art is within the scope of the present...

Claim
... one of a tablet, liquid, gel, cream, ointment,, spray, and lotion.
SU19STITUTE,9j4EeT.

32 A **system** for providing localized MoSsbauer absorptions and selective release of energy in an organic medium, comprising said source occurs in the Mossbauer absorber atom.

33 The **system** of claim 32 wherein said source comprises one of a magnetized ferromagnetic source, a quadrapole split source and a filtered source,

34 The **system** of claim 32 wherein said means for conforming comprises:
means for providing a gradient magnetic...

...incident energy at a selected location within said organic media.
SUBS UTE SHEET

35 The **system** of claim 34, wherein said field gradient comprises field lines varying from substantially colinear with...

...field line within the range of varying field lines which permitg Mossbauer absorptions.

36 The **system** of claim 35,, wherein said means for conforming sequentially provides field lines of radial, transverse...

...a plane parallel relative to said

incident gamma rays., within sàld-organic media.

37 The **system** of claim 36, wherein said means for conforming; includes a pair of Helmholtz coils having...

...of said Helmholtz
coil in opposition to the other of said Helmholtz coil,
38 The **system** of claim 36., wherein said means for conforming includes:
a plurality of Helmholtz coil's...

...two coils
having a current flow in mutual opposition,
SUBST"UTE SMEEr
3 9, The **system** of claim 32,, wherein said filtered source includes means for separating wanted from unwanted electromagnetic radiation.
4 0 The **system** of claim 35,, wherein said means for separating includes a crystaline diffraction grating.

41 The **system** of claim 32, wherein said'source of gamma rays comprises a tunable energy gamma ray source,

42 The **system** of claim 41., wherein said source of gamma rays comprises a synchrotron source providing gamma rays of selected energy levels.

43 The **system** of claim 32,, wherein said means for conforming comprises means for providing acoustic energy to one of said organic media and said source.

44 The, **system** of claim 43, wherein said means for providing acoustic energy.provides ultrasound energy,

45 The **system** of claim 43, wherein said means for providing acoustic energy provides said acoustic energy along...

...rays at a selected target location in said organic media.
SU8'MTLrrE SHEET

46 A **process** for providing spatially localized Mossbauer absorption in an organic medium, comprising the steps of:
selectively...absorption of the applied gamma rays by said selectively disposed Mossbauer absorber atom.;

47 The **process** of claim 46, wherein said step of applying comprises applying a gamma ray with a monochromatic line.
4S, The **process** of claim 46, wherein said step of conforming includes, providing a gradient magnetic field of...

...to the applied gamma rays. at a selected location within said-.organic media.

49 Th@=. **process** of claim 46,, wherein the step of conforming comprises the step of applying acoustic energy...

...coincide with the gamma
ray energy at the selected location.
RI IRCOVENG"D

50 The **process** of claim 49, wherein the step of applying an acoustic energy comprises applying ultrasound energy.

51 A **process** for providing spatially localized energy absorption in an organic medium of a biological **system** , comprising the steps of:
administering a compound. containing a Mossbauer absorber atom which -is selectively uptaken to a selected location within said organic medium of said biological **system** ;
applying gamma ray energy from a source to the location of selective uptake in said...

...absorber
atom at the selected locations,, providing absorption of the gamma rays therein.

52 The **process** of claim 51 wherein ' the Mossbauer absorber atom comprise bone seeking Mossbauer absorber atoms, including...

...Gd, 1 5 7 Gd, 161 Dy., 1 61 Dy and
149' Sm,

53 The **process** of claim 51 wherein the step of administering a Mossbauer absorber atom comprises administering a compound containing a Moss,bauer' absorber atom,

54 The **process** of claim 51, wherein the step of administering comprises the step of administering a compound containing a Mossbauer absorber atom having a selected molecule bound thereto,
SUBSTITUTESHEET
55.. The **process** of claim 54, wherein said molecule comprises at least one of:
a monoclonal antibody,, a hormone,, a derivatizing functionality,, a catonic lipophilic dye, a colloid, and an aggregatemblecule.

56 The **process** of claim 55, wherein said derivatizing functionality includes hematoporphryin and bleomycin.

57 The **process** of claim 54,, further including the step of binding one of the Mossbauer absorber atom...

...molecule to a portion of - t@e organic media at the selected location,

58 The **process** of claim 51, wherein the Mossbauer resonance of said Mossbauer absorber atom differs from said applied gamma rays, the **process** further including the step of:
conforming the Mossbauer resonance characteristics energy of said Mossbauer absorber...

...Mossbauer
absorption of the - applied gamma rays by said administered-Mossbauer absorber atom.

59 The **process** of claim 58, further including the step of interacting the Mossbauer absorber atom with the...

...hyperfine interaction and quadrapole interaction of the Moszbauer absorber atom nucleus,
SUBSTrrLrre SHEEr

60 The **process** of claim 58, wherein the step of conforming comprises the step of applying a magnetic...

...applied gamma
rays, permitting gamma ray energy absorption by said Mossbauer absorber atom.

61 The **process** of claim 58, wherein the step of conforming comprises the step of applying acoustic energy...

...absorber atom to coincide with the gamma ray energy at the selected location,

62 The **process** of claim. 61, wherein the step of applying an acoustic energy comprises applying ultrasound energy.
63* A **process** of providing energy absorption at a selected target tissue in a biological **system** , comprising the steps of:
administering a Mossbauer absorber atom to said biological **system** wherein the uptake of the Mossbauer absorber atom in the target tissue provides a locally...

...of
said Mossbauer absorber atom, permitting gama ray absorption therein,
SUBST'rLrrE SHIMET

64 A **method** of using the compound of claim 1 for medical diagnosis or treatment., comprising the steps of:
administering an effective amount of the compound to a biological **system** ; and
selectively applying a selected frequency electromagnetic radiation to the biological **system** to
ode Mossbauer absorption of said electromagnetic
provi
radiation at selected target areas within said biological **system** .,

65 The **method** of claim 64 wherein said electromagnetic radiation comprises gamma rays.
66 The **method** of claim 64., 'wherein said step of administering compri .ses at least one of intravenoust intramuscular, subcutaneous, intra-arterial and intra-articular injection of said compound.
67., The **method** of claim 64, wherein said step of
P
administering comprises at least- one of topical application and oral administration..

68 The **method** of claim 64, wherein said step of selectively applying comprises employing electromagnetic radiation at a dose effective to eliminate cell lines causing selective **necrosis** at said target areas,

69 The **method** of claim 64, wherein said biological **system** comprises an animal;
said target area comprises a **cancer** ; and
s@aid Mossbauer absorption by said compound causes **cancer** **necrosis** .
SUBST'TUTE SHEET
PCTIUS88/01796 . 199 -
70 The **method** of cl aim 69t wherein said animal comprises a human*
J,
'r
SUBSTI-I UT SHEET
APPARATUS PROVIDING DIAGNOSIS
AND SELECTIVE TISSUE **NECROSIS**
CROSS REFERENCE TO RELATED APPLICATIONS
This application is a continuation-in-part of my co...

...Mossbauer effect for
diagnostic and therapeutic purposes.
BACKGROUND OF THE INVENTION
In the treatment of **tumors** by ionizing radiation, typically X-rays or gamma rays are used, The ideal in **radiation** therapy of **malignant** disease is achieved when the **tumor** is completely eradicated, and the surrounding normal tissue, in the treated volume, shows little or...

...or
functional injury. The important factor in successful treatment is the difference in radiosensitivity of **neoplastic** and normal cells. All tissues, normal and **neoplastic** , are affected by radiation so that radiosensitivity is a relative term, The basic consideration of **radiation** **therapy** is that cells that are actively proliferating or that cells which are of a primitive...

...tissue so that there is usually
a considerable margin between doses that are damaging to **neoplastic** and to normal cells. If this is the case, then a multifraction dose schedule decreases the size of the **tumor** over time while permitting time

SUBSTITUTE SHEET
between doses for normal tissue to recover, A constant fraction of **tumor cells** are **killed** with each treatment, and theoretically the **tumor** can be completely eliminated with a sufficient number of treatments. However, normal tissue has a...

...s history is eventually reached. Exceeding this threshold results in unacceptable side effects. Thus, the **tumor** volume must be reduced sufficient ly before the threshold is reached or the **cancer** is incurable by this modality of therapy.

SUMMARY OF THE INVENTION

The present invention is pharmaceuticals, apparatus,, and a **process** which provides diagnosis, therapy and other biological effects by use of highly selective absorption of...analogously to the dispersion device in optical absorption. By varying the driving velocity,, a resonance **system** can be driven by the emitted gamma photons with regard to the nuclear energy transitions...

...biological target such as the DNA of the target tissfie as part of a therapeutic **process** . Alternatively, the present invention provides diagrams by monitoring the release . of nonlethal energy, as described...polarization and propagation direction conditions to achieve resonance in the MIRAGE absorber pharmaceutical, The Apparatus, **Systems** , Compounds, **Methods** , and specifications of use are described in detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

These...

...detailed description taken together with the drawing, wherein:

Fig* 1 is one embodiment of the **system** apparatus of the present invention;

Fige 2 is an alternate embodiment of the **system** apparatus of the present invention;

Fige 3 is an alternate embodiment of a portion of the **system** of Figs. 1 or 2., showing the position of surface coils;

Fig, 3A is a...

...isometric view of an alternate embodiment of an array of coils for use in the **system** apparatus of Figs, 1 and 2,;

SUE STITUTEE SHEET

Fig. 7 is an isometric drawing of a **system** according to the present invention showing ultrasound modulation of the gamma ray source and the...

...at the target area;

Figs* 8 and 9. are graphical plots of data related to **radiation therapy** ;

Figs. 10A, B and C are diagrammatic representations of the MIRAGE pharmaceutical 12/ ...a 3/2-+1/2 transition

in an oriented absorber with a unique principle axis **system** ; and
Figs, 17A and B are the spectra from a single crystal of a-Fe...

...300 K,
SUCM-SMTUTE 9SSHEET
DETAILED DESCRIPTION OF THE INVENTION
The present invention includes the **process** of producing pharmaceuticals having desired Mossbauer nuclear parameters such that they possess physical and chemical...

...the target tissue via the Mossbauer effect.
The pharmaceuticals of the present invention and the **process** of producing the pharmaceuticals is discussed first, which is followed by the apparatus used in combination with selected --pharmaceuticals to effect the Mossbauer absorption in a biological target as a **process** of the invention to provide a therapeutic or diagnostic function. The latter, apparatusq provides a...the pharmaceutical molecules present in the target selected tissue,
SUBSTITUTE SHEET
- 10
Implementation of the **process** for making MIRAGE pharmaceuticals involves selecting an atom responsive to the Mossbauer effect at a...includes a polarizing element, to polarize the
emission. Polarized gamma rays are obtained by three **methods** : magnetized ferromagnetic. sources, quadrapole split sourcesF or filter **techniques** . In addition, the apparatus possesses means to produce external magnetic fields and ultrasonic beams toSUBSTITUTE SHEET
The **process** of providing selectivity by imparting magnetic f ields with the apparatus involves providing a magnetic...

...of the nonselected tissue to the gamma rays and absorption by the selected tissue,
The **process** of treatment involves using the pharmaceuticals and apparatus in combination to cause the Mossbauer effect...

...and - direction is applied,
or an ultrasonic beam is applied. For the ultrasonic case, the **process** of effecting selectivity by causing an ultrasonic beam to intersect the administered gamma ray beam...by changing the direction of the source polarization magnetic field in the case of a **ferromagnetic** source),, the magnetic **field** strength gradient (e.g. by changing the current in the surface coils which give rise...the source of fluorescence is used in a feedback loop which feeds into* a control **system** which changes the magnetic field strength and direction; ultrasonic beam frequency, direction and power; and...

...or past experience.
A representative calculation of an effective photon flux for treatment to achieve **necrosis** and the associated dose appears in the Theoretical Section as does the theory of achieving selectivity by the modes mentioned. (Implicit is that the **process** for diagnosis is the same as that for treatment with regards to excitation. Detection is...

...Testing Of MIRAGE Treatment
Using MIRAGE Drug 12/29/w
The human colon and breast **cancer** cell lines., HT29 and MCF7, respectively, were obtained from Cambridge Research Lab Inc., and were...Lab Inc.,, which the Kundsin Lab tested for
these organisms. The human breast and lung **cancer** cell lines, HTB26 and A549,, respectively, were obtained from the American Type Culture Collection. The...
...normalized to that of the control,
RESULTS
The effects of lm rad levels of Mossbauer **radiation** absorbed during MIRAGE **treatment** of the **cancer** cell lines MCF7, McCoy, HT29, HTB26, and A549 using the MIRAGE drug 12/19/w...for the elimination of a pathological cell population. Previous experiments demonstrated that the most potent **killing** effect in **cells** by radiation is from secondary particles produced by internal conversion of gamma ray energy followed...

...nontoxic levels of radiation which are six orders of magnitude less than that of conventional **radiation** **therapy** where the Mossbauer effect was exploited for treatment, The ability to control the occurrence of...Ory
A / A
@b
A 5b
ary
SUBSTITUTE S FEE T
- 51
(Organometallic Com-Pounds **Methods** of Synthesis ,Physical - Constants and Chemical Reactions., Michael Dubb., Editor, 2nd Edition, Vole III, (1968...with NaOH and copper bronze to
yield the antimony derivatized acridine 19 according to the **method** of O'Donnell, G.J., Iowa State Coll, J. Sci,,, 20,, 34-6 (1945); CA...dilithium naphthalene 39, is reacted with tellurium to yield the product 40 according to the **method** of Marfat, A., et al, Journal of the American Chemical Society, 99't (1977) ppe...53, is reacted with trimethyllithium germanide 15,, to give the product 55,, according to the **method** described in Qmprghensive Organs Me-t-allic @Qhemistry, Sir Geoffrey Williams, Editor (1982) Vol. 2...hydroxyquinoline which intercalates DNA directly,
90 is synthesized using the indicated Mossbauer isotopes by the **procedures** referenced in The Actinide Elements, Kew* Bagnall, (1972) pp. 211-229,

incorporated by reference,
A3q 101, which is
prepared according to the **method** described in
Comprehensive Organometallic Chemistry, Geoffrey
Wilkinson, Editor, (1982), Vol. 4, Po 1018
(incorporated by...compound 130, is reacted with
iridium adduct 131 to give the product 132 by the
procedure described by Gardner, S.A., et al, Journal
of Organometallic Chemistry, 60 (1973) 179-188j...

...reacted with diazonium
adduct 134 to give the o-metallated adduct 135
according to the **method** of Farrell, N,; et al,
Journal of the Chemical Society, Dalton, Trans.1
1977j, 2124...

...with Grignard
reagent 139 followed by chlorination to give chloride
adduct 140 according to the **procedure** of Rausch, M.D.
and Moser, G.A., Inorganic Chemistry, Vol. 13, No. 1,
1974...

...is reacted with phosphine
compound 147 to give o-metallated adduct 148
according to the **procedure** described in Comprehgnsive
Organometallic ...and by
isolating the product by filtration of evaporation of
the solvent using reactions and **techniques** generally
known to one skilled in the art.
For example,, sodium thiosulfate is treated with...protecting vials and
may be
refrigerated if necessary.
THE APPARATUS
The overall operation of the **system** may be
exemplified by the C057 /Fe 5 7Mossbauer pair as
follows: the radioactive source...changing the ultrasonic driving
frequency.
The source, or emitter of radiation,, can also
include the **techniques** known to Mossbauer
spectroscopy of narrowing the line width or absorbing
unwanted Mossbauer lines. In...in Fig, 1 to
give a characteristic plot of the treatment
effectiveness. A spatially distributed **system** of
multiple detectors such as proportional counters or
SUBSTMUTIESHEET
- 120
scintilation detectors, or lithium drifted...
...intensity of
treatment, A control signal can be derived from the
fluorescence, and combined or **processe** -d by
processor 94 of Fig. 1 according to the orientation
of detectors which record signal direction and...

...relative to the patient as shown in
Fig* 2 and transverse to the patient, A **system** of
such Helmholtz coils are used as described below to
effect the field characterstics necessary...so that resonant absorption
can be
localized to specific dimensions (such as that of a
tumor) while maintaining nonresonant, and therefore

nonabsorptive, conditions in the surrounding
nonselected tissue at the energy...rays' propagation
direction, and selective absorption will occur for
the Am = 0 line by the **process** described in the
Theoretical Section,
An alteration of this scheme is to use two pairs...realized
selectivity by polarization and energy mechanisms
discussed in the Theoretical Section.
In a preferred **method** where fields are used to
achieve selectivity, treatment is carried ...common to both the path 212
of the
gamma rays and the beam 206 of **acoustic** energy.
Treatment can be controlled by a microprocessor
which receives digitized input from peripheral
sensors wh

ich...

...SURVY ITUrE SHEET
- 130
of magnitude greater than the processing times of
high speed control **systems** .
ADDITIONAL APPLICATIONS
MIRAGE drugs and therapy have many diverse
applications in addition to the treatment of **cancer** .
For example, MIRAGE compounds can be used for imaging
and for treatment of any disorder...

...used in scintiscans to gain diagnostic
information based on the physiological properties of
the pathological **process** . These properties include
differential uptake,, concentration,, or excretion of
the radionucleotide by normal versus diseased...by hepatocytes, and the
gallium
scan, in which the radionuclide 67 Ga is
concentrated in **neoplastic** or inflammatory cells to a
greater degree than in hepatocytes, Hence,, a
sues I TTUTIE...

...or "hot spot" with a gallium scan. The
gallium scan is also helpful in diagnosing **neoplastic**
infiltration in the patient with cirrhosis, since the
tumor will show increased uptake, while fibrous bands
will show decreased uptake. Another major
application of...

...on differential
uptake, excretion, or concentration as a consequence
of the physiology of the pathological **process** , But..
Mossbauer scintiscans also provide the ability to
diagnose disease **processes** and to selectively image
different tissues based on the phenomenon of the
differential resonance frequency...
...function of distance along the
source axis, a correction algorithm has to be used to
process the data to produce an image of. the actual
distribution of the mossbauer isotope or...

...AUTOIMMUNE, AND
TRANSPLANTATION REJECTION DISEASE

A successful treatment for rheumatoid arthritis
is the induction of **necrosis** of synovial cells of
afflicted joints. For example, intra-articular
radioactive synovectomy using the radionucleotide...

...reducing
inflamation, effusion and pain in patients with
rheumatoid arthritis,
MIRAGE therapy provides selective cellular
necrosis and intra-articular MIRAGE synovectomy can
be substituted for intra-articular radioactive
synovectomy to give...

...and
by substituting stable Mossbauer absorber isotopes
for radioactive 1 6 5Dy in the synovectomy
treatment , systemic **radiation** exposure from leakage
is avoided.
Ferric hydroxide macroaggregate is massive in a
recoil sense and...

...Mossbauer radiation is administered to
the joints.
Other diseases which can be cured by inducing
necrosis of specific cell lines include autoimmune
diseases and transplantation rejection disease which
includes graft versus...

...such as
carboxyl, amino, sulfide.. halogen,, or carbonyl and
condensing the two entities together by **methods**
generally known to one skilled in the art, The
protein binds to surface of the...

...and involved in
atherosclerosis,
The occlusion of arteries is the end result of
the atherosclerotic **process** which involves the
following stages 1) repeated injury which denudes the
vessel of endothelium, 2 which can **kill** **cells** which have incorporated
the drug
by using levels of radiation which pose no threat to...

...000 deaths per year which compares
with 30,,000 deaths per year due to breast **cancer** ,
AIDS is a fatal disease with no specific treatment,
and development of a vaccine presents...

...from other human -pathogenic viruses
because it destroys the T cell segment of the immune
system which normally is responsible for controlling
SUBST7TUTE SHEET
- 136
the elimination of a viral challenge...

...cytopathic.
Also, the biology of the virus is such that it can
elude the immune **system** during a latent phase and
then activate to produce virus at a tremendous rate
before the host **cell** **dies** . This life cycle is a

consequence of a transactivating factor.. tat III,, and trs, a...may require rapid viral protein synthesis and assembly in the race between virion release and **cell death** ,. The presence of large amounts of tat III at the time of a trs-mediated...

...enzymes can only slow the relentless progress of this disease which destroys the hostes immune **system** by a T cell cytopathic life cycle. The viral message exists in the host DNA...

...in an infected individual is to destroy all such cells before the host's immune **system** is inundated with virus and irreversibly compromised. MIRAGE drugs represent agents which can selectively discriminate...

...environment at the Mossbauer atom of an intercalating MIRAGE drug can be exploited as a **method** to selectively eliminate HIV infected cells in the latent stage, Tat III is the only...

...drug followed by systemic irradiation at the frequency of the created isomer shift will selectively **kill** latent infected **cells** and interrupt the infectious **process** .

THEORETICAL SECTION

PRINCIPLES OF **RADIATION THERAPY**

Ionizing **radiation** was found shortly after its discovery to be capable of reducing the growth of human **tumors** , Unfortunately the limitations of this modality were discovered as patients developed catastrophic late complications. The such that the balance of these opposing ends is in favor of **tumor** ablation. The total story of. the cellular mechanisms involved remains elusive; however, many of the...

...basic understanding of the effect of radiation on cells and the cellular response to damage, **Radiation therapy** involves particle and electromagnetic radiation which causes damage to both normal and **cancer** tissue. The goal is to ablate the **tumor** while preserving normal tissue. The principles involved are manifested in cell survival curves, In Fig...

...m2 =
*9 where 3 doubling times. Cells
SUB,qj I tUTE SHEET
- 139
exposed to **radiation** reach a **treatment** threshold and then are killed exponentially, the survival number versus radiation dose is an exponential curve where a constant fraction of the **cells** are **killed** per treatment. All **tumors** can be controlled as 'the dose goes to infinity; however,, it is the limitation of tolerance of normal tissue not the ability to c@ntrol the **tumor** which is the guide to treatment, Thus,, it can be appreciated that a significant factor...

...rate equation below:

N o(D os e. (6)
Critical is a reduction of the **tumor** burden,, -N., to a level which is no longer overwhelming to the body's natural defenses.
Treatment with **radiation** can lead to a cure even though this is a local modality which has no effect on distant micrometastases despite the shedding of **malignant** cells by **tumors** which are below the mass sufficient for diagnosis. Current data supports three explanations for this...

...host has the ability
to kill a limited number of viable metastatic cells.
(3) The **tumor** mass influences its own metastatic potential. **Radiation therapy** by diminishing the mass reduces the source of clonogenic metastases and increases the host's ability to deal with
SUBSTMM SMSET
- 140
residual micrometastases by eliminating the **tumor** 's adverse effect on the host immune **system** , The ideal in **radiation therapy** of **malignant** disease is achieved when the **tumor** is completely eradicated and the surrounding normal tissue of the treatment volume is structurally and...

...intact, The important factor in the successful treatment is the difference in the radiosensitivity of **neoplastic** and normal cells which is the slope,, Ml of equation 6. The difference depends on...damaged. In general, if surrounding tissue can tolerate twice the radiation dose of a given **tumor** ,, then the **tumor** is radiosensitive. Alternately, a **tumor** which extensively involves both lungs, and may be cured with a dose of 3000 rads, cannot be treated effectively with **radiation therapy** because of the greater radiosensitivity of the surrounding lung tissue.
All **tumors** can be eradicated by **treatment** with sufficient **radiation** . But,, damage to normal tissue is dose limiting due to the acute and late effects of **radiation therapy** , Acute effects include esophagitis, pneumonitis, and diarrhea. They occur Z shortly after treatment and limit...

...limiting in radiation. They
often progress with time and are usually irreversible, These include fibrosis, **necrosis** , fistula formation, non-healing ulcerations, and damage to specific organs such as spinal cord transection...

...as a guide to long term ef fects, There are a number of examples in **radiation therapy** where the total dose has been increased,, the size of the dose f raction increased...

...due
to depletion of the stem cell pool, Acute effects depend on the balance between **cell killing** and

compensatory replication of both the stem and proliferative compartments. The development of late effects...

...that the stem cells have only a limited proliferative capacity. Compensation for extensive or repeated **cell death** may exhaust this capacity resulting in ...into irradiated mice until they lose the ability to reconstitute the recipient's marrow.
Successful **radiation therapy** can be understood from the dynamics of cellular responses to radiation. From the dynamic point of view, the basic difference between a normal renewal tissue of the body and a **tumor** is that in normal tissue there is an effective balance between cell production and cell loss; whereas, in **tumors** , cell proliferation exceeds cell loss. The normal renewal tissue can be considered a hierarchy of three types of cells: Stem cells -4 Maturing cells -+ Functioning cells.
The cell cycle of **cancer** cells are in general shorter than those of normal tissue, It is found in general that irradiation causes an elongation of the generation cycle of **tumor** cells while a corresponding shortening of the cell cycle of normal cells is the norm...

...is enhanced if post radiation conditions are suboptimal for growth. Both of these mechanisms favor **tumor** cells over normal cells.
Thus, a major factor leading to a cure and which underlies...

...dose regimens that are so commonly employed"@ in clinical radiotherapy.
As with normal tissue,, different **tumors** have a range of radiosensitivity-some being responsive to a few hundred rads, and others...

...as much as 10,000 rads, and this variation can even exist within a specific **tumor** type. Furthermore, radioresistanc6 is selected for in the **tumor** population as normal tissue regenerative capability declines. Thus, it can be appreciated, from survival curves...

...in Figs. 8 and 9., that necessary but not sufficient conditions for a cure via **radiation therapy** are that the- first order kinetics of **cell kill** must be sucly that enough **cancer cells** are **killed** and the **tumor** does not return to its original mass in the time interval necessary for normal tissue to regenerate, And,, the **tumor** volume is reduced to a level which can be eliminated by the host's defenses...

...which will ultimately produce unacceptable late:

effects..
SUES I if U TIE SHEET
PHYSICS OF **RADIATION THERAPY**
Ionizing **radiation** exerts its effects on atoms
primarily as a function of the number of electrons.
Biological...but
lacks the ability to repair double strand breaks
which is the lethal event in **radiation therapy** .
The **radiation** effects on particular molecules
such as DNA,, are ascribed to two **processes** ,, direct
and indirect action, By direct action is meant the
effects of energy directly in...probability as demonstrated by the
inverse
relationship between the number of decay events
needed to **kill** a given **cell** type bya radioisotope and
the number of radiated electrons which it produces.
SUBSTTTUTE SHEET
For...

...electron which is then
ejected as an Auger electron to produce a new
vacancy, The **process** continues shell by shell, until
the valance shell is reached and thus leads to
multiple...an Auger cascade
which cause radiolysis and double strand breakage is
lethal to a cell, **Radiation therapy** is far less
efficient requiring approximately 105 photons
absorption events per cell to-produce the...

...radioactive atoms and with electromagnetic radiation
doses one million times less than that of
conventional **radiation therapy** . This is accomplished
by utilizing phenomenon common to electromagnetic
radiation therapy and radioactive atomic DNA
labeling, MIRAGE therapy entails using Mossbauer
atomic labeled pharmaceuticals which bind...

...for the case of 1 2 5 I
labeled DNA, Furthermore, this single event will
kill the target **cell** which is in contrast to
conventional **radiation therapy** where multiple
improbable events must occur simultaneously to
produce a double strand break, 105 photons...

...needed for MIRAGE therapy,
The absorption cross-section for water the primary
target of conventional **radiation therapy** is
approximately 10@25 cm 2 whereas the resonant
cross-section for Mossbauer absorption is 10- 17
cm2 which represents an eight order of magnitude
improvement. This increased efficiency permits **cell**
kill with radiation

doses of one millionth that of
conventional therapy,
PHYSICS AND CHEMISTRY OF MIRAGE...pharmaceutical
molecule that permits the use of this phenomenon to
selectively treat disease such as **cancer** .
The Mossbauer effect is degraded by recoil
energy of the emitted and absorbed photon. This...the recoilless or

recoil-free
fraction. To increase the relative strength of the
recoilless resonant **process** ,, it is important that f
be as large as possible. The recoilless fraction f
can...

...molecule.
As described previously, Auger cascades in DNA
binding pharmaceuticals cause DNA radiolysis and
concomitant **death** of the **cells** in the target tissue,
The equation which relates the number of internal
conversion events with...respectively,
SUIRSTffUTS 814EET
Table 8
alpha
B= beta Representative Mossbauer Isotopes with Parameters Favorable for
Cancer Therapy
Half Life **Gamma** Half Life /Auger Mossbauer
of Ground Isotope Ray or Excited (Cross- Line Ret
State(yr...levels and well below levels that are necessary to
cause acute or late effects of **radiation therapy** .
Furthermore, MIRAGE **therapy** is a modality whereby the
side effects of chemotherapy can be eliminated,
MIRAGE drugs are isotopes from
Table 7 appear in the Exemplary Material Section.
SELECTIVITY
Selective **killing** of selected **cells** with. sparing
of nonselected cells can be achieved by several
mechanisms:
SUIRS T ITUTE SHEET...

...the side bands.
For case 1
IRAGE therapy can achieve selectivity in the
case of **cancer** therapy in animals including humans
via exploiting known selective uptake by **cancer** cells
of compounds such as Bleomycin, cationic lipophilic
dyes such as Rhodanine, hematoporphryins, and
monoclonal...

...bound to the
compound known to be selectively taken up by the
SUBST"VT
EISHEET
cancer , In contrast to chemotherapy, the selectivity
need only be relative to other bell types in...

...such as
carboxyl, amino, sulfide, halogen, or carbonyl and
condensing the two entities together by **methods**
generally known to one skilled in the art,
Colloids such as those of gallium are known to
be concentrated by certain types of **cancer** cells and
the same phenomenon is predicted for certain colloids
of Mossbauer isotopes comprising massive...

...incorporated into biological matrices including bone
which is useful for the treatment of metastatic bone
cancer , Examples include 40 Ko is 3 Gd/
1 6 1Dyj, 16 3YI 1 4gM J...the precursor molecules of

thyroid hormones. All can serve as targets for treatment of thyroid **cancer** with MIRAGE therapy. And 57 Fe can be incorporated into heme proteins and red blood...

...frequency of deoxyhemoglobin which differs from that of oxyhemoglobin to exploit the relative hypoxia of **tumors** where hypoxia results in a greater concentration of deoxyhemoglobin. Furthermore, damage to the red blood cells in the **tumor** leads to coagulation followed by thrombosis of the blood supply to the **tumor** and concomitant **tumor** death, I 5 For case 2
The energies of the nuclear states are weakly inf...result of the
presence of an internal magnetic field which can be generated by an **unpaired electron** in the **atomic** environment that can induce an imbalance in electron spin density at the nucleus or by...realized in the selected cells which is different from that of nonselected cells. For example, **cancer** cells are known to have differences in ion concentrations and ph from normal cells. Binding...only for the proper spin moment alignment. Polarized gamma rays can be obtained by three **methods** , magnetized ferromagnetic sources, quadrapole split sources, or filter **techniques** as shown by U. Gonser and H. Fischer, Current Topics in the Physics of Mossbauer Spectroscopy, The Exotic Side of the **Method** : Resonance Gamma Ray Polarimetry, 99-135; incorporated by reference.
Selectivity via polarization of the source...

...radiation and its dependence on orientation are determined by conservation of angular momentum in the **system** of nucleus plus gamma ray (quantum selection rules) where the quantum-mechanical - **treatment** of electromagnetic **radiation** leads to the introduction of photons which are bqsons of vanishing rest mass and quantized...the Am = 0 lines becomé strong,
Selective eradication of a selected cell line such as **cancer** tissue can be achieved by polarizing the **cancer** tissue with an orientation different from surrounding normal tissue and by irradiating with radiation which...

...referring to Figs, 17A and B, the nuclei of the MIRAGE pharmaceutical present in the **cancer** tissue can be aligned perpendicularly to the propagation direction of the gamma ray; whereas, the...By irradiation with
gamma rays which are resonant with the Am = 0 transition, only the **cancer** tissue will absorb the radiation.
SUE3811 rUTE SHEET
- 175
For Case 5
The line shape...in the absence of the Mossbauer effects. The equation for determining the

total dose from **gamma** ray **treatment** and the depth of penetration of the photons appears in Table 11,
SUBSTITUTE SHEET
I...

...radiation using a miniturized source and mass drive or ultrasonic drive. Breast,, bowel., and pancreatic **cancer** are candidates for the former; and lung **cancer** is a candidate for the latter, Mossbauer sources of high energy gamma rays which penetrate deeply can be used to treat **tumors** that are not located superficially, 155 Gd is the source of a 60 KeV Mossbauer...

...cm2/gm and represents
a suitable source for the treatment of primary and metastatic bone **cancer** and deep solid **tumors** .
SUBSTMJTE SHEET
ja3Hs 3jLnju.Lssns
a% tA -A@, W hj =mmo=
=Q== 0 00 a...125 DAUGHTER IS STABLE*
SUBSTITUTE SHEET
- 184
Modifications and substitutions of the compounds, pharmaceuticals, apparatus, **methods** , **systems** , and **process** steps made by one skilled in the art is within the scope of the present...one of a tablet,, liquid, gel, cream, ointment,
spray, and lotion,
SUBSTITLME,gMSET

32 A **system** for providing localized Mossbauer absorptions and selective release of energy in an organic medium, comprising...of the gamma rays from said source occurs in the Mossbauer absorber atom.

33 The **system** of claim 32 wherein said source comprises one of a magnetized ferromagnetic source, a - quadrapole split source and a filtered source.

34 The **system** of claim 32 wherein said means for conforming comprises:@
means for providing a gradient magnetic...

...a
selected location within said organic media.
SUES I I I UTE SHEET

35 The **system** of claim 34, wherein said field gradient comprises field lines varying from substantially colinear with...

...field line within the range of varying field lines which permits Mossbauer absorptions.
36 The **system** of claim 35,, wherein said means for conforming sequentially provides field lines of radial, transverse...

...a plane parallel relative to said
incident gamma rays, within said organic media.

37 The **system** of claim 36, wherein said means for conforming includes a pair of Helmholtz coils having...

...of said Helmholtz
coil in opposition to the other of said Helmholtz
coil.

38 The **system** of claim 36, wherein said means for conforming includes:
a plurality of Helmholtz coils having...

...at least two coils
having a current flow in mutual opposition,
SURaTn'JMSHIEEr
39 The **system** of claim 32, wherein said filtered source includes means for separating wanted from unwanted electromagnetic radiation.

40 The **system** of claim 35, wherein said means for separating includes a crystaline diffraction grating.

41 The **system** of claim 32, wherein said source of gamma rays comprises a tunable energy gamma ray source.

42 The **system** of claim 41, wherein said source of gamma rays comprises a synchrotron source providing gamma rays ot selected energy levels.

43 The **system** of claim 32, wherein said means for conforming comprises means for providing acoustic energy to one of said organic media and said source.

44 The **system** of claim 43, wherein said means for providing acoustic energy provides ultrasound energy.
45 The **system** of claim 43., wherein said means for providing acoustic energy provides said acoustic energy along...

...rays at -a selected target location in said
organic media.
8UB'MTLrre,SHE

46 A **process** for providing spatially localized Mossbauer absorption in an organic medium, comprising the steps of:
selectively ...absorption of the applied gamma rays by said selectively disposed Mossbauer absorber atom,

47 The **process** of claim 46, wherein said step of applying comprises applying a gamma ray with a monochromatic line.

48 The **process** of claim 46, wherein said step of conforming includes providing a gradient magnetic field of...
...to the applied gamma rays

at a selected location within said organic media.

49 The **process** of claim 46, wherein the step of conforming comprises the step of applying acoustic energy...

...coincide with the gamma ray energy at the selected location,
SUBSTITUTE SHEET
- 195

50 The **process** of claim 491 wherein the step of applying an acoustic energy, comprises applying ultrasound energy,

51 A **process** for providing spatially localized energy absorption in an organic medium of a biological **system** , comprising the steps of:
administering a compound containing a Mossbauer absorber atom which is selectively uptaken to a selected location within said organic medium of said biological **system** ;
applying gamma ray energy from a source to the location of selective uptake in said...

...absorber atom at the selected locations, providing absorption of the gamma rays therein,

52 The **process** of claim 51 wherein the Mossbauer absorber atom comprise bone seeking Mossbauer absorber atoms,, including...

...155 Gd, 15 7 Gd, 161 Dyj, 16 3 Dy and 149 Sm,

53 The **process** of claim 51 wherein the step of administering a Mossbauer absorber atom comprises administering a compound containing a Mossbauer absorber atom,

54 The **process** of claim 51.. wherein the step of administering comprises the step of administering a compound...

...a Mossbauer absorber atom having a selected molecule bound thereto.
SUBSTITUTE-SHEET
- 196

55 The **process** of claim 54., wherein said molecule comprises at least one of:
a monoclonal antibody, a...

...a derivatizing functionality, a catonic lipophilic dye, a colloid, and an aggregate molecule.

56 The **process** of claim 55, wherein said derivatizing functionality includes hematoporphryin

and bleomycin.

57 The **process** of claim 54,, further including the step of binding one of the Mossbauer absorber atom...
...the molecule to a portion of the organic media at the selected location.

58 The **process** of ...the Mossbauer resonance of said Mossbauer absorber atom differs from said applied gamma rays, the **process** further including the step of:
conforming the Mossbauer resonance characteristics energy of said Mossbauer absorber...

...Mossbauer absorption of the applied gamma rays by said administered Mossbauer absorber atom.

59 The **process** of claim 58, further including the step of interacting the Mossbauer absorber atom with the...

...interaction and quadrapole interaction of the Mossbauer absorber atom nucleus,
SUBST'TUTE S146ET
60 The **process** of claim 58, wherein the step of conforming comprises the step of applying a magnetic...

...gamma
- rayst permitting gamma ray energy absorption by said Mossbauer absorber atom. R

61 The **process** of claim 58, wherein the step of conforming comprises the step of applying acoustic energy...

...absorber atom to coincide with the gamma ray energy at the selected loclation.

62 The **process** of claim 61, wherein the step of applying an acoustic energy comprises applying ultrasound energy.

63 A **process** of providing energy absorption at a selected target tissue in a biological **system** , comprising the steps of:
administering a Mossbauer absorber atom to said biological **system** wherein the uptake of the Mossbauer absorber atom in the target tissue provides a locally...

...resonance of said Mossbauer absorber atom, permitting gama ray absorption therein,
SUBvTnrLrrE SHEEr

64 A **method** of using the compound of claim 1 for medical diagnosis or treatment,, comprising the steps of:
administering an effective amount of the

compound to a biological **system** ; and
selectively applying a selected frequency electromagnetic radiation to the biological **system** to provide Mossbauer absorption of said electromagnetic radiation at selected target areas within said biological **system** , P

65 The **method** of claim 64 wherein said electromagnetic radiation comprises gamma rays*
66 The **method** of claim 64,, wherein said step of administering comprises at least one of intravenous,, intramuscular, subcutaneous, intra-arterial and intra-articular injection of said compound.

67 The **method** of claim 64, wherein said step of administering comprises at least one of topical application and oral administration.

68 The **method** of claim 64, wherein said step of selectively applying comprises employing electromagnetic radiation at a dose effective to eliminate cell lines causing selective **necrosis** at said target areas.

69 The **method** of claim 64,, wherein said biological **system** comprises an animal;
said target area comprises a **cancer** ; and
said Mossbauer absorption by said compound causes **cancer** **necrosis** ,

70@ The **method** of claim 69, wherein said animal comprises a human.

CORRECTED VERSION*

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :		(11) International Publication Number: WO 88/ 09152
A61B 19/00	A1	(43) International Publication Date: 1 December 1988 (01.12.88)

(21) International Application Number: PCT/US88/01796

(22) International Filing Date: 27 May 1988 (27.05.88)

(31) Priority Application Number: 055,591

(32) Priority Date: 28 May 1987 (28.05.87)

(33) Priority Country: US

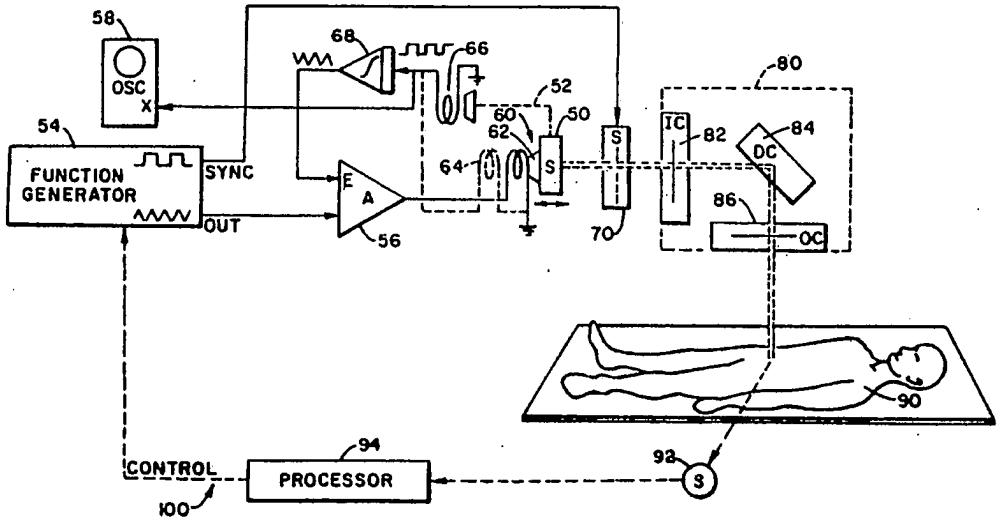
(71)(72) Applicant and Inventor: MILLS, Randell, L. [US/US]; R.D. 2, Cochranville, PA 19330 (US).

(74) Agents: MATZUK, Stephen, G. et al.; Weingarten, Schurgin, Gagnebin & Hayes, Ten Post Office Square, Boston, MA 02109 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), BR, CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent), SU.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: APPARATUS PROVIDING DIAGNOSIS AND SELECTIVE TISSUE NECROSIS



(57) Abstract

Pharmaceuticals and apparatus used in combination for diagnosis and tissue necrosis applicable to provide effective and selective therapy using the Mossbauer absorption phenomenon. Selected pharmaceutical compounds containing a radiation absorber isotope are administered to a tissue and excited by a radiation source (50) which provides energy at the corresponding resonant Mossbauer absorption frequency of isotope containing pharmaceutical, where excitation effects nuclear transitions to cause highly selective energy absorption in the selected target tissue. For diagnostic purposes, de-excitation fluorescence of the isotope is monitored. For therapeutic purposes, the energy is converted to particle radiation by the isotope at the target tissue by internal conversion followed by an Auger election cascade which results in radiolysis of DNA resulting in lethal double strand breaks in the DNA molecules of the target tissue. The tissue selectivity is achieved by providing a Mossbauer absorption frequency of the target tissue which differs from that of surrounding tissue.

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis
Volume 372, Issue 1 , 11 November 1996, Pages 23-31

doi:10.1016/S0027-5107(96)00105-4 Cite or Link Using DOI

A 3 milliTesla 60 Hz magnetic field is neither mutagenic nor Co-Mutagenic in the presence of menadione and MNU in a transgenic rat cell line

Andrew Suri[a], Johan deBoer[a], Wolfgang Kusser[a] and Barry W. Glickman[a, *]

[a] Centre for Environmental Health, Department of Biology, University of Victoria, P.O. Box 3020 Victoria, B.C., V8W 3N5 Canada

Received 7 August 1995; revised 20 May 1996; accepted 29 May 1996. Available online 30 November 1999.

Abstract

The mechanisms by which an electromagnetic field (EMF) influences biological material are poorly understood. One potentially important model suggests that a magnetic field can stabilize free radicals in such a way as to permit their dispersement rather than their return to the ground state (Okazaki et al., 1988, Scaiano, 1995). We have tested this hypothesis by examining mutagenesis in the *E. coli lacI* gene target carried in the Big Blue® rat embryo fibroblast cell line, R2λLIZ. Mutant frequencies were determined in cells exposed to a magnetic field, cells pretreated with the mutagens*N* -methylnitrosourea (MNU) or 2-methyl-1,4-naphthoquinone (menadione), prior to being held in a 60 Hz 3 milliTesla (mT) magnetic

field and cells concurrently exposed to the mutagens and the magnetic field. Menadione was selected because its mutagenic mechanism involves the formation of free radicals, while MNU is an alkylating agent not thought to act through radical formation. According to the radical stabilization hypothesis the application of a magnetic field to menadione treated cells would accentuate the mutagenic effects. Our results failed to indicate that the magnetic field affects mutagenesis by the oxygen-radical mediated mutagen, menadione.

---

*Corresponding author.

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis
Volume 372, Issue 1 , 11 November 1996, Pages 23-31

25/3,K/2 (Item 2 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

11

00535635 **Image available**
APPARATUS AND METHOD FOR INTERFERING WITH PATHOLOGICAL CELLS SURVIVAL PROCESSES
APPAREIL ET PROCEDE PERMETTANT D'ENTRAVER LES PROCESSUS DE SURVIE DE CELLULES PATHOLOGIQUES

Patent Applicant/Assignee:
TOFANI Santi,
Inventor(s):
TOFANI Santi,

Patent and Priority Information (Country, Number, Date):
Patent: WO 9966987 A1 19991229
Application: WO 99EP4385 19990623 (PCT/WO EP9904385)
Priority Application: EP 98830381 19980624
Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 7421

APPARATUS AND METHOD FOR INTERFERING WITH PATHOLOGICAL CELLS SURVIVAL PROCESSES
APPAREIL ET PROCEDE PERMETTANT D'ENTRAVER LES PROCESSUS DE SURVIE DE CELLULES PATHOLOGIQUES

Main International Patent Class: **A61N-002/02**
Fulltext Availability:
Detailed Description
Claims

English Abstract
A **method** and an apparatus for interfering with pathological cells survival **processes** , i.e. inducing directly or indirectly **apoptosis** , on living pathological cells, by using magnetic fields without adversely affecting normal cells. Static (S...

...a field frequency comprised between 1 and 1000 Hz. An apparatus for carrying out the **method** comprises means for generating static magnetic (S) fields crossing a working environment and/or means...

French Abstract
La presente invention concerne un procede et un appareil permettant d'entraver les **processus** de survie de cellules pathologiques en induisant directement ou indirectement l' **apoptose** de cellules pathologiques vivantes a l'aide de champs magnetiques, sans entrainer d'effets indesirables...

Detailed Description
TITLE
APPARATUS AND **METHOD** FOR INTERFERING WITH PATHOLOGICAL CELLS SURVIVAL **PROCESSES**
DESCRIPTIO
Field of the invention
The present invention generally relates to an apparatus for interfering with pathological cells survival **processes** .

In addition, the invention relates to a microbiological **method** carried out by such apparatus for interfering with pathological cells survival, in particular cells affected by **cancer** and other diseases caused by alterations in the mechanism of cell survival.

In particular, the interference is induced by means of static (S) and extremely low frequency **electromagnetic** (ELF) **fields** produced by the apparatus.

Magnetic Static fields and Extremely Low Frequency **electromagnetic fields** are hereinafter referred to also as S and ELF, respectively. Moreover, any possible combination of...

...It is known that pericellular fields and currents induced by an Extremely Low Frequency (ELF) **electromagnetic field** , whose frequency range is from 1 Hz to 300 Hz and perhaps up to 1000...

...cell certain membrane electrochemical events which are important for primary biologic signal transduction and amplification **processes** .

These biochemically mediated events then produce cytoplasmic second messengers and internal effectors such CONFIRMATION COPY...

...1991, 9Blanchard
19941.

The possibility of influencing variations of Ca" metabolism may lead to cell **apoptosis** (programmed **cell death**) [loPreston,"Trump 19971 .

Another physical interaction mechanism is related to the possibility of influencing the...

...cell signalling pathways of the cell (including calcium metabolism) through a field direct effect on **electron** -spin motion of **atoms** and molecules with **unpaired electrons** . This influencing may affect the recombination ratio of a spin correlated **free radical** pair and consequently on redox signalling [12 Grundler 1992; 13 Polk 1992; 14 Walleczek and...

...1992; 15 Adey
19931.

In particular, the spin singlet-triplet energetic level transition in a **free radical** is critical for increasing the recombination ratio of spin correlated **free radical** pairs.

The possibility for low level, non thermal (with intensity up to 30 mT) S...

...kinetics and efficacy of radical pair reactions is known from magnetochemistry ["Steiner

1989] .

Naturally occurring **free radicals** have an oxygen or nitrogen-based ...ROS) and Reactive Nitrogen Species (RNS) can target proteins providing an obvious mechanistic explanation for **free radicals** -mediated signalling events.

These events may influence growth factors, ion transport (i.e. Ca" channels), transcription, **apoptosis** ["Lander 19971.

Apoptosis is a morphologically distinct form of programmed **cell death** that is connected in cell survival **processes** playing an important role during development, homeostasis, and in many diseases including **cancer** , acquired immunodeficiency syndrome, and neurodegenerative disorders, as well as in other diseases that similarly to those are characterised by altered cell survival **processes** . **Apoptosis** occurs through the activation of a **cell** -intrinsic **suicide** program. The basic genetic mechanism of **apoptosis** appears to be present in essentially all mammalian cells at all times, but the activation...

...originate from both the intracellular and the extracellular environment.

Among all the genes involved in **apoptosis** regulation, the p53 gene is receiving much attention.

This gene, which encodes a transcription factor and is common in many human **cancers** , mediates the cellular responses to some environmental damage. The p53 protein either can temporarily stop...

...so that the cell can repair altered DNA, or can pilot the cell to an **apoptotic** death.

Published data support that p53 appears in **apoptosis** through a three step **process** : 1) transcriptional induction of redox-related genes: 2) the formation of reactive oxygen species and 3) the oxidative degradation of mitochondria components, culminating in **cell death** [18Polyak 19971 .

In addition anti-oxidative agents are combined with drugs in the treatment of hypoxia **tumour** cells " [Walch, 19881 and in the influence of vascular growth factor "[Amirkhosravi, 19981.

Moreover, published...

...fields in leukaemic lymphocytes but not in normal lymphocytes 22 Walleczek, 19961.

Altered cell survival **processes** come with electric disorders and different electrical behavior. In fact, rapidly proliferating and transformed cells...

...Marino 1994]. It has also
been shown that epithelial cells lose their
transepithelial potential during **carcinogenesis** ["Davies
1987; 26 Goller 1986 27 Capko, 19961. This different
electrical behavior of **tumor** cells compared with normal
cells is the basis for a newly proposed **cancer** diagnostic
modality [28CUZ ick 19981. In addition, the concentration of
free radicals in transformed cells and tissues is higher
than in non-transformed ones [21S zatrowski 1991...

...19951.

With reference to chemotherapy all efforts are
devoted to the target of inducing cell **apoptosis** in vivo
instead of killing them, through Signal Transduction
Directed Therapy (STDT) of **cancer** [12 Levin, 19981.

Signal Transduction is a functional term that connotes
the translation of genetic...

...external stimuli and/or duplicate itself. Recent
evidence suggests that alterations in the cell survival
processes contribute to the pathogenesis of a number of
human diseases, including **cancer** , viral infections,
autoimmune diseases, neurodegenerative disorders, and AIDS.

Treatments designed to specifically alter the **apoptotic**
threshold connected with the survival **processes** mechanisms
may have the potentiality to change the natural progression
of some of these diseases ["Thompson, 19951.

High intensity electrical, **electromagnetic** and
magnetic **fields** have been used to destroy pathological
cells.

In 14 US4665898 an apparatus is described in which
animals having **malignant** cells are treated by means of a
high intensity pulsed magnetic field, in order to
neutralise/destroy **malignant** cells in a selective way.

This apparatus produces magnetic thermal fields having
intensity comprised between...

...and
250 Kilohertz.
Different ELF, thermal, continuous or pulsed fields,
have been used for anti- **cancer** therapy in vitro [3'Narita,
1997; 36Raylman, 19961 .

In these cases the fields are of...

...ELF low intensity electromagnetic f ields have been
used as well to inhibit mitosis of **malignant** cells, such
as in DE 412238OAl and US 5156587. However, these
documents describe the use...

...Summary of the invention
It is an object of the present invention to provide
a **method** for interfering with cell survival **processes**

(i.e. inducing **apoptosis**) of living pathological cells (i.e. **cancer** cells) by using magnetic fields without adversely affecting normal cells.

It is another object of the invention to provide an apparatus for interfering with pathological cells survival **processes** .
The f ormer and other objects are reached by the **method** for interfering with pathological cells survival according to the invention whose characteristic is to apply to living pathological cells (i.e. **cancer** cells and cells affected by other diseases caused by alterations in the mechanism of cell survival) non thermal SELF magnetic fields to induce **apoptosis** in a selective way.

For the purposes of the invention SELF fields are to be fields alone.

The concept underlying the **method** according to the invention is that SELF fields interfere with cell signalling sustaining cell pathological behaviour inside pathological cells, i.e. on redox signalling through **free radicals** , thus restoring the cell survival **processes** , i.e. inducing directly or indirectly **apoptosis** through a modification of p53 gene expression.

This **method** is supposed to recombine oxygen-based **free radicals** and may also be used as an anti-oxidative agent. It's combination with drugs in the treatment of hypoxia **tumour** cells and in the influence of vascular growth factor may also be considered.

The reason why SELF fields selectively induce **apoptosis** in pathological cells (i.e. **cancer** cells) may be related to the altered electrical behaviour of pathological cells compared with that...

...normal cells.

For these reasons SELF fields can induce directly or indirectly a signal programmed **cell death** (**apoptosis**), in vitro and in vivo, without causing any adverse effect.

In the hypothesis that **free radicals** recombination is at the basis of the expected biological effects on pathological cells (i.e., anti- **tumour** activity) the transition between singlet-triplet of unpaired electron in oxygen based **free radicals** has to be considered. In fact this transition, which depends on the applied magnetic field, is critical for increasing the recombination ratio of a spin correlated **free radical** pair. However, the reaction centres related to the expected anti **tumor** effect are unknown and therefore the lifetime of the spin states and the energy splitting...

...for
reaching optimal condition(s) for the singlet-triplet spin state conversion required for the **free radical** recombination **processes** [1 3Polk 1992].

For these reasons, S, ELF or SELF fields have higher probability to...

...to another aspect of the invention, an apparatus for selectively interfering with pathological cells survival **processes** in vitro and in vivo has the characteristic of comprising means for generating static magnetic...ELF, S+ ELF fields can be produced 5 that can also be repeated cyclically.

The **method** according to the invention will now be described in more detail by way of specific examples.
EXAMPLE 1
In this experiment the capability of inducing **apoptosis** by SELF magnetic field as a function of field intensity and frequency was studied in...

...field was maintained constant.

After 3 hours the cells were treated with May- Grunwald Giemsa. **Apoptosis** was assessed by counting the number of **apoptotic** nuclei per 10 high power fields (HPF) by using an optic microscope.

The amount of induced **apoptosis** was evaluated by the ratio between the number of **apoptotic** cells found in the exposure group and the number of **apoptotic** cells found in the shame-exposed group, that is the group not exposed to the...

...obtained in different exposure conditions.

- 13
TABLE 1
exposure SELF field frequency field intensity (Static + **apoptosis**
conditions composition (Hz) ELF rms) mT ratio
A S (static) (0.5 + 0) 1
B...

...highly significant (at the t Student test). From Table 1 we can see that the **apoptosis** effect appears at 2 mT and doubles starting from 3 mT.

Another important finding is that **apoptosis** doesn't depend upon SELF field frequency. In other words during the lifetime of the mechanism operating the biological effect (**apoptosis**) the ELF field is seen as essentially constant. This means that between the two hypothesised mechanism, **free - radicals** (occurring in a time scale of nano- to microsecond) and ion resonance-like mechanisms, the **free radical** one is playing the role ["Scaiano, 1994, 4OEngstrom, 19971.

EXAMPLE 2
In this experiment the selective effect of SELF magnetic fields was verified exposing three cell lines.

Two lines were **malignant** , human colon adenocarcinoma cells

5 (WiDr) and human breast **cancer** cells (MCF-7). The normal cell line was human lung fibroblast (MRC-5).

As in...

...3
exposed and three shame-exposed) for each cell line were exposed for 20 minutes. **Apoptosis** was evaluated after 3 hours. The exposure conditions used were the R type of Table 1.

The results are reported in Table 2.

TABLE 2
cell line **apoptosis** ratio
WiDr 2.1
MCF-7 1.4
MRC-5 1
As shown in Table 2 only **cancer** cells reported an **apoptosis** increment statistically highly significant, whereas the normal cell line didn't. The difference in percentage of **apoptosis** between the two **cancer** cell lines was expected due to the two different duplication times.

In fact WiDr duplicates...

...at t Student test.

EXAMPLE 3
In this example nude mice (nu/nu) bearing subcutaneous **tumour** masses were used to assess the influence of SELF magnetic fields on **tumour** growth inhibition.

Each mouse was inoculated subcutaneously with 10 million human colon adenocarcinoma cells (WiDr...

...kV/m was also
applied to eventually take advantage of the different electrical behaviour between **tumoral** and normal tissues
42
['Thornton, 1984 ; Barsamian, 1987]
In the second experiment 24 female mice...

...shame-exposed.

All the mice of both experiments were divided into experimental groups after the **tumor** masses for each animal were palpable.

The animals were exposed for 70 minutes, once a...

...by N.I.H. (US National Institute of Health) and N.C.I. (US National **Cancer** Institute).

The **tumor** masses were measured twice a week and their volume calculated in mm' according to the...

...diameter) x (minor diameter squared)] / 2.

After 4 weeks the animals were sacrificed and autopsied. **Tumor** masses were extracted, weighed and measured. Portions of **tumors** were used for different analysis, i.e.

- immunoistochemical: Ki-67 antigen for proliferative index, ...staining for the assessment of number of mitosis;
- ultrastructural: electron microscopy;
- nucleic acid hybridisation: Tunel **method** for **apoptosis** evaluation.

In addition, the following organs were extracted from each animal for histologic examination to...

...axillary and inguinal limphonodes, mediastinal limphonodes, ovaries, skin, spleen, bone marrow, subcutaneous tissue (site of **tumoral** cell line implantation) as well as blood tests.

The obtained results are reported in Table...

...field alone 50% 50% 50% 0%
number of mice 6 6 6 6 12
extracted **tumor** mass volume 1323 1450 920 650 1492
(MM) +/- 304 288 540 205 559
extract **tumor** mass weight (g) 1.54 +/- 1.6 +/- 0.98 0.96 1.6 +/- 0.5
0.22 0.39 0.56 0.25
number of **apoptotic** cells per 1 0 98 +/- 115 129 129 40
HPF 23 20 25 26 17...

...exposure conditions 4
(see tab. 3) shame exposed
number of mice 12 1 2
extracted **tumor** mass volume 1139 +/- 509 CM3 1914 +/- 793 CM3
extracted **tumor** mass weight 1.4 +/- 0.7 g 2.1 +/- 0.6 g
apoptosis (assessed in 50% of mice 72.5 +/- 9.3 37.0 +/- 7.4
only)
p53...

...The data reported in tables 3 and 4 show that SELF fields have an inhibitory **tumor** growth effect in vivo.

This effect, found in both experiments, was statistically
5 highly significant...
...related to the SELF fields treatment.

The ultrastructural analysis by electron microscope showed in the **tumor** cells of exposed animals many cellular alterations: presence of **apoptotic** bodies and condensed chromatin near the nuclear membrane characteristic of **apoptotic** events.

In addition a consistent result is represented by morphological modifications, increase of number and...

...mitochondria as well as number of nucleoli, presence of many vacuoles inside the cytoplasm. Non **neoplastic** cells (i.e. epithelial and stromal cells) showed no differences between exposed and shame-exposed...

...of toxicity found in
12 normal organs examined in each animal.

- 18
The increment in **apoptosis** as well as the decrement in p53 gene expression found in exposed mice **tumors** (see tables 3 and 4) are statistically highly significant (t Student test)
Results reported in...

...and 2.

The effect induced by the SELF magnetic fields on p53 expression enforces the **apoptosis** results and is in agreement with the hypothesised biophysical mechanism io (i.e. **free radical** recombination) by which the SELF fields have an anti- **tumor** effect through formation of reactive oxygen species and the degradation of mithocondrial components.

EXAMPLE 4...

...for 5 days a week, for their entire
life beginning after 24 hours after the **tumor** inoculation.

The exposure conditions were the same of the
experiment the results which are reported...R, Shirley-Henderson A (1991): "Transcription
and Translation in Cells exposed to Extremely Low
Frequency **Electromagnetic Fields** " Bioelectrochem.

Bioenerg. 25, pp. 335
-' Phillips jl, Haggren w, Thomas WJ, Ishida-iones T and...

...Cyclotron resonance in membrane
transport. In Chiabrera A, Nicolini C., Schwan HP (eds).

"Interactions Between **Electromagnetic Fields** and Cells".

New York: Plenum Press, pp 281
Chiabrera A., Grattarola M., Viviani R. (1984).

"Interaction between **electromagnetic fields**0 and cells.

Microelectrophoretic effect on ligands and surface
receptors". Bioelectromagnetics 5, ppl73
I Lednev VV (1991) : 'Possible mechanism for the influence
of weak magnetic fields on biological **systems** ".

Bioelectromagnetics 12, pp 71
Blanchard JP, Blackman CF (1994):"Clarification and
application of an ion param etric resonance model for
magnetic field interactions with biological **systems** .

Bioelectromagnetics 15, pp217
10 Preston GA, Barrett JC, Biermann JA and Murphy Elizabeth
(1997) : "Effects of Alterations in Calcium Homeostasis on
Apoptosis during **Neoplastic** Progression", **Cancer** Research
57, pp. 537

Trump BF, Berezesky IK, Chang SH and Phelps PC (1997) :"The Pathways of **Cell** Death : Oncosis, **Apoptosis** , and **Necrosis** ". Toxicologic Pathology Vol. 25, n. 1, pp 87.

11 Grundler W, Kaiser F, Keilmann F, Walleczek J (1992) "Mechanisms of electromagnetic interaction with cellular - 21
systems "..Naturwissenschaften 79, pp. 551
13 Polk C (1992):"Dosimetry of extremely-low-frequency magnetic fields...Related Phenomena". Chem. Rev.

89, pp. 51
11 Lander HM (1997):" An essential role for **free radicals** and derived species in signal transduction". The FASEB Journal 11, pp118
18 Polyak K, Xia Y, Zweier JL, Kinzier KW and Volgestein B (1997):"A model for p53-induced **apoptosis** ". Nature Vol.

389, pp. 300
'9 (18). Walch, N.S., Calaoagan, J., Murphy, B.J...

...R. "The redox sensitive human antioxidant responsive element induces gene expression under low oxygen conditions".

Carcinogenesis , 19 (8): 1333-7,1988.

'oAmirkhosravi, A., Meyer, T., Warnes, G., Amaya, M., Malik, Z...

...P., Siddiqui, F.A., Sherman, P., Francis, J.L. Pentoxifylline inhibits hypoxia-induced upregulation of **tumor** cell tissue factor and vascular endothelial growth factor. Thromb Haemost, 80 (4): 598-602, 1998...

...A, Zucchini P, Emilia G, Torelli G and Claudio Franceschi (1992):"Lymphocytes and low-frequency **electromagnetic fields** ". The FASEB Journal Vol. 61 pp 2674.

Walleczeck J (1996):" **Electromagnetic Field** Effects on Cellular Signal Transduction and **Free Radical** Mechanisms".

Abstract Book XXVth General Assembly of the International Union of Radio Science-Lille-France...

...547.

"Binggeli R, Weinstein RC. Membrane potentials and sodium channels: hypotheses for growth regulation and **cancer** formation based on changes in sodium channels and gap junctions. Theor Biol 1986: 123:377...

...Schwalke MA, Gonzales E, Marler KC, Flanagan CA. Association between cell membrane potential and breast **cancer Tumour** Biol. 1994: 15:82 - 22
25 Davies RJ, Weidema WF, Sandle GI, Palmer LI, Deschener EE, DeCosse JJ. Sodium transport in a mouse model of colonic **cancer** . **Cancer** Res. 1987: 47:4646

26 Goller DA, Weidema WF, Davies RJ. Transmural electrical potential as an early marker in colon **cancer** . Arch. Surg.

1986: 121:345
27 Capko D, Zhuravkov A, Davies RJ. Transepithelial depolarisation in breast **cancer** . Breast **Cancer** Res. 1996.

Treat. 41:230.

" Cuzick J, Holland R. , Barth V, Davies R, Faupel M...

...Sacchini
V, Vanel D, Veronesi U. Electropotential measurements as a new diagnostic modality for breast **cancer** . The Lancet 1998: 352:359
29 Szatrowski TP, Nathan CF. Production of of large amounts of hydrogen peroxide by human **tumor** cells. **Cancer** Res.

1991: 51 (3):794
31 Shulyakovskaya T, Sumegi L, Gal D. In vivo experimental studies on the role of **free radicals** in photodynamic therapy. I. measurement of the steady state concentration of **free radicals** in **tumor** tissues of mice. Biochem.

Biophys. Res. Commun. 1993: 195 (2):581
31 Iwagaki H, Hamazaki K, Matsubara N, Hiramatsu M, Orita K, Mori A..Lipid peroxidation in hepatocellular **carcinoma** .

Acta Med. Okayama 1995: 49 (6):313
11 Levin VA (1998) :"Signal Transduction Directed Therapy.

Fact or Fantasy?" Abstract Book (EL 5) of the Eight International Congress on Anti- **Cancer** Treatment, February 3rd -6 th 1998, Paris, France.

13 Thompson C.B. (1995) :" **Apoptosis** in the pathogenesis and treatment of diseases" Science Vol. 267, p. 1456-1462
34 Costa JL and Hofmann GA (1987) : " **Malignancy** treatment" U.S. patent 4,665,898.

35 Narita K, Hanakawa K, Kasahara T, Hisamitsu T, Asano K (1997) :"Induction of **apoptotic cell death** in human leukemic cell line, HL-60, by extremely low frequency electric magnetic fields: analysis...

...AC, Wahl RL (1996):"Exposure to Strong Static Magnetic Field Slow the Growth of Human **Cancer** Cells In Vitro". Bioelectromagnetics 17, pp. 358
37 Haberkorn R, Michel-Beyerle ME. On the...

...N, Cozens FL, McLean J and Thansandote (1994):"Application of the Radical Pair Mechanism to **Free Radicals** I Organized **Sy**stems : Can the Effects of 60 Hz Be Predicted From Studies Under Static Fields?" Bioelectromagnetics 15...

...554.

40 Engstrom S (1997) :"What is the Time of Magnetic Field Interaction in Biological **Systems** ?". Bioelectromagnetics

18, pp. 244
41 B.S. Thornton (1984) "Inversion of raman spectra of living...
...198202.

42 S.T. Barsamian (1987): 'Dielectric origin of living cells", in Biophysical Aspects of **Cancer** , Charles University Prague, pp. 152-159
- 24

Claim
1 Apparatus for selectively interfering with pathological cells survival **processes** in vitro and in vivo characterised in that it comprises:
- means for generating static magnetic...

...1 and 1000 Hz versus time.

2 Apparatus for selectively interfering with pathological cells survival **processes** in vitro and in vivo characterised in that it comprises:
- means for generating static magnetic...

...predetermined function of intensity versus time.

3 Apparatus for selectively interfering with pathological cells survival **processes** in vitro and in vivo characterised in that it comprises:
- means for generating electromagnetic extremely...fields for selectively interfering with pathological cells survival, such as in particular cells affected by **cancer** , viral infections, autoimmune diseases, neurodegenerative disorders, AIDS, etc., characterised in that said SELF non thermal...

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 966 988 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
29.12.1999 Bulletin 1999/52

(51) Int. Cl.[6]: **A61N 2/02**

(21) Application number: **98830381.4**

(22) Date of filing: **24.06.1998**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(71) Applicant: **Tofani, Santi**
10010 Burolo (To) (IT)

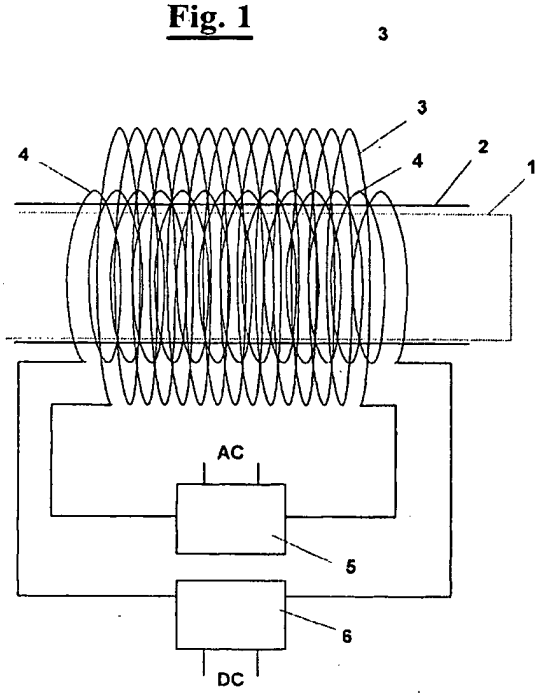
(72) Inventor: **Tofani, Santi**
10010 Burolo (To) (IT)

(74) Representative:
Celestino, Marco et al
ABM, Agenzia Brevetti & Marchi,
Via A. Della Spina 40
56125 Pisa (IT)

(54) Apparatus and method for interfering with pathological cells survival

(57) A method and an apparatus for interfering with pathological cells survival, i.e. inducing apoptosis on living pathological cells, by using magnetic fields without adversely affecting normal cells. Static (S) and extremely low frequency (ELF) magnetic fields are used having intensity comprised between 1 and 30 mT. In particular SELF fields are used which are different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz. An apparatus for carrying out the method comprises means for generating static magnetic (S) fields crossing a working environment and/or means for generating electromagnetic extremely low frequency (ELF) fields over the working environment in addition to the S fields. Means are provided for modulating the S fields and varying the intensity of the S fields from 1 to 30 mT. Means may also be provided for modulating the ELF fields associated to the ELF fields generating means and imposing to the ELF fields a frequency between 1 and 1000 Hz with intensity comprised between 1 and 30 mT.

Fig. 1



EP 0 966 988 A1